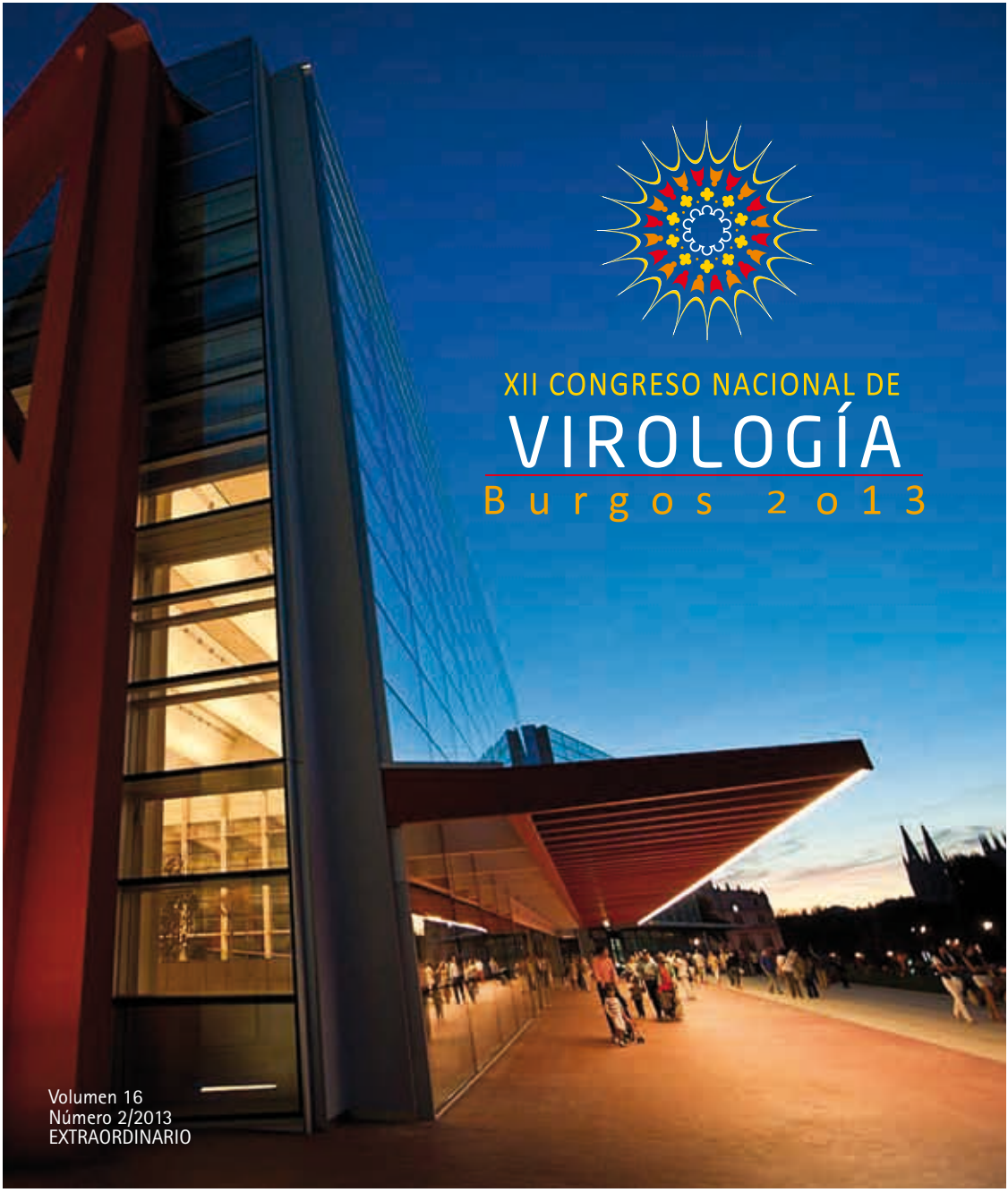
Sociedad Española de Virología

VIROLOGÍA

Publicación Oficial de la Sociedad Española de Virología

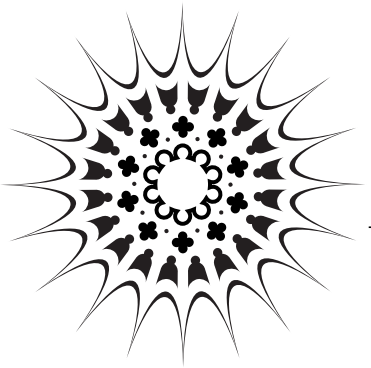


XII CONGRESO NACIONAL DE VIROLOGÍA

Burgos 2013

Volumen 16
Número 2/2013
EXTRAORDINARIO

VIROLOGÍA. PUBLICACIÓN OFICIAL DE LA SOCIEDAD ESPAÑOLA DE VIROLOGÍA



XII CONGRESO NACIONAL DE VIROLOGÍA

Del 9 al 12 de junio

Aula Magna de la Universidad de Burgos y

Fórum Evolución Burgos

Burgos 2013

Volumen 16
Número 2/2013
Extraordinario

Edición y Coordinación:
Ana Doménech Gómez

Diseño y Maquetación:
Eventtual [info@eventtual.net]

Fotografía Portada:
Luis Mena

Impresión:
Imprenta Provincial de Burgos

Deposito Legal:
BU 171-2013

ISSN (versión impresa):
1133-0384

ISSN (versión digital):
2172-6523

Centro de Biología Molecular "Severo Ochoa"
C/ Nicolás Cabrera, 1
28049 Cantoblanco - Madrid

sev@cbm.uam.es

Página web del Congreso:
www.cab.inta-csic.es/congresovirologiasev2013

XII CONGRESO NACIONAL DE
VIROLOGÍA

Estimados amigos y colegas:

La Sociedad Española de Virología (SEV) y el Comité Organizador os damos la bienvenida al XII Congreso Nacional de Virología (XII CNV), que se celebra en Burgos del 9 al 12 de junio de 2013. Las sedes del XII CNV son el Aula Magna de la Universidad de Burgos en la sesión inaugural del día 9, y el Palacio de Congresos Fórum Evolución Burgos en las sesiones de los días 10 al 12.

Gracias al trabajo realizado por los miembros del Comité Organizador y del Comité Científico, y gracias también a la participación de todos los que habéis enviado comunicaciones, hemos conseguido un atractivo programa que cubre los diferentes campos de la virología básica y aplicada, en sus vertientes humana, veterinaria y vegetal. Además, exploraremos la fecunda interacción de la virología con otras áreas de la biología (en particular, el estudio de la evolución), así como con la medicina, la química y la física. De esta forma, esperamos que todos los investigadores básicos y clínicos que trabajáis en cualquiera de las ramas de la virología participéis activamente en este Congreso. Asimismo contamos con la presencia, como ponentes invitados, de varios virólogos nacionales y extranjeros de reconocido prestigio. Entre todos podremos discutir sobre los avances, logros y retos en el estudio de los virus, en un Congreso que esperamos sea muy fructífero. Además, hemos preparando un interesante programa social y cultural que incluye la visita a dos de los principales atractivos científicos de Burgos: los yacimientos de la Sierra de Atapuerca y el Museo de la Evolución Humana.

Queremos agradecer al Presidente y a todos los miembros de la Junta Directiva de la SEV el apoyo que nos han prestado para la organización de este Congreso. También agradecemos la ayuda de la Universidad de Burgos y del Hospital Universitario de Burgos en los aspectos locales de la organización. Por último, expresamos nuestra gratitud a las demás instituciones y a todas las empresas que colaboran en el XII CNV como patrocinadores: en una época de profunda crisis como la que estamos atravesando, este Congreso no habría sido posible sin su apoyo.

Esperamos que durante estos días todos disfrutemos tanto del XII CNV como de los variados atractivos de Burgos y su entorno.

Recibid un cordial saludo en nombre del Comité Organizador,

Carlos Briones
Presidente del Comité Organizador del XII CNV

XII CONGRESO NACIONAL DE
VIROLOGÍA

Dear friends and colleagues:

The Spanish Society for Virology (SEV) and the Organizing Committee are pleased to welcome you to Burgos on the occasion of the XII Spanish National Congress of Virology (XII CNV). The Congress is held at the Aula Magna of the University of Burgos (opening session, June 9) and at the Congress Center Fórum Evolución Burgos (June 10-12 sessions).

Thanks to the work carried out by the members of the Organizing and Scientific Committees, and to all the participants who have submitted their abstracts to this Congress, we have set up an interesting program covering various basic and clinical aspects of human, veterinary, and plant virology. We will also explore the fruitful interaction of virology with other areas of biology (in particular, the study of evolution), as well as with medicine, chemistry and physics. Therefore, we are confident that basic and clinical researchers working in any branch of virology will be willing to actively participate in this Congress. In addition, we are both pleased and honored to host several renowned Spanish and foreign virologists as invited speakers. All together, we will be able to discuss current progresses, achievements, and challenges in the study of viruses. We have also prepared an interesting social and cultural program that includes a visit to two of the major scientific landmarks of Burgos: the Archaeological Site of Atapuerca and the Museum of Human Evolution.

We wish to thank the President and the Executive Board of the SEV for their support to this Congress. We also appreciate the help of the University of Burgos and the Hospital Universitario de Burgos on local aspects of the organization. Finally, we gratefully acknowledge the sponsorship of several institutions and companies: in the deep crisis we currently face, the XII CNV would not be possible without their support.

It is our hope that we all enjoy not only the Congress but also the varied attractions of Burgos and its surroundings.

With kind regards, on behalf of the Organizing Committee,

Carlos Briones
Chairman of the Organizing Committee of the XII CNV

SIN CIENCIA NO HAY FUTURO

Nos encontramos en Burgos celebrando el XII Congreso Nacional de Virología, auspiciado por la Sociedad Española de Virología, y dentro de dos años estaremos compartiendo nuestro XIII Congreso. Al menos ese es el deseo de todos nosotros. Que podamos volver a reunirnos depende, en gran medida, de las actuaciones del actual Gobierno de España. La Ley de la Ciencia aprobada hace dos años (Ley 14/2011) no se ha implementado aún. La Agencia Estatal de Investigación promovida por dicha Ley, uno de cuyos objetivos era garantizar un marco estable de financiación y favorecer la internacionalización de nuestros grupos, está fracasando antes de su puesta en marcha. La financiación de nuestro sistema científico lleva camino de perder una anualidad completa, retrasando la subvención de los proyectos concedidos en la convocatoria de 2012 y aplazando la siguiente convocatoria, que tendría que haberse publicado a finales del pasado año. Lo mismo se puede decir de las contrataciones de personal científico, tanto los contratos indefinidos como los de incorporación o de personal técnico. Y de las ayudas para la realización de Congresos. Perdemos también en internacionalización si el Estado no paga las cuotas que nos permiten participar en convocatorias y grandes instalaciones europeas. En estas condiciones, ¿podemos asegurar la continuidad de nuestros equipos y de nuestros proyectos?, ¿cómo podemos mantener un sistema que no se renueva ni financia?, ¿podemos ser competitivos?

La actividad científica es necesaria tanto para aumentar el conocimiento como para incidir positivamente en el desarrollo económico y social de un país. Así, es evidente que los estados que poseen un sólido sistema de Ciencia y Tecnología no viven la actual crisis del mismo modo que nosotros. La necesidad de cambiar el modelo productivo de España es ya un clamor dentro y fuera de nuestras fronteras. De hecho, organizaciones nacionales e internacionales, de las que se hace eco un reciente editorial en la revista *Nature*, han urgido a nuestro país a invertir mucho más en Ciencia como una medida imprescindible para salir de esta crisis... y para poder afrontar mejor las que vengan en el futuro. En 1632, el dramaturgo y poeta Lope de Vega escribió "Obras son amores y no buenas razones" en su novela dialogada *La Dorotea*. Poco aprecian y respetan nuestros gobernantes a la sociedad española si las "obras" que promueven en el sistema de I+D+i son las que padecemos en la actualidad. Sus "obras" serán las que permitan o impidan a las generaciones futuras el cambio necesario. Es su responsabilidad.

Sociedad Española de Virología y
Comité Organizador del
XII Congreso Nacional de Virología

NO SCIENCE, NO FUTURE

We have met in Burgos to attend the XII National Congress of Virology, organized by the Spanish Society for Virology. Within two years, we should be sharing our XIII Congress. Or, at least, this is the wish of all of us. Whether or not this wish is accomplished depends largely on the policy of the current Spanish government. The Science Act (Law 14/2011) was approved two years ago, but it has not been implemented yet. The Research Agency that was promoted by such a Law, two of whose objectives were to ensure a stable financial framework for our science and to facilitate the internationalization of our groups, is failing before commissioning. The scientific system is losing a full annuity as a result of the delay in the funding of projects granted in 2012, and the postponing of the subsequent annual call, due by now. Analogous delays affect the calls for scientific staff, including permanent and temporary contracts, as well as for technical staff. Moreover, financial support for the organization of scientific meetings was discontinued two years ago. Internationalization of our science is also hampered for the reason that the Spanish government does not comply with the fees that would allow us to participate in European calls and large international facilities. Under these conditions, can we ensure the continuity of our research teams and our projects? How can a system that is neither renewed nor financed survive? Can Spanish science be competitive?

Scientific activity is necessary both to increase our knowledge and to impact positively on the economic and social development of any country. Thus, those countries with a strong Science and Technology system do not suffer the current crisis in the same way as we do. The Spanish productive model is in need of a deep change, as it is increasingly recognized inside and outside our borders. In fact, national and international organizations, recently echoed through an editorial in *Nature* journal, have urged our government to increase investment in science as an unavoidable measure to overcome this crisis, as well as to better face the upcoming ones. In 1632, the Spanish playwright and poet Lope de Vega wrote, "Actions speak louder than words" in his work *La Dorotea*. Our leaders would show little care and respect for the Spanish society if the "actions" they undertake for promoting R&D&I are those we experience today. Their "actions" will either grant or deny future generations the required change. This is our leaders' responsibility.

Spanish Society for Virology and
Organizing Committee of the
XII Spanish National Congress of Virology

COMITÉS / COMMITTEES

COMITÉ ORGANIZADOR / ORGANIZING COMMITTEE

Presidente / Chairman:
Carlos Briones Llorente.
(Centro de Astrobiología, CSIC-INTA, Madrid)

Secretario / Secretary:
Josep Quer Sivila
(Hospital Universitari Vall d'Hebron, Barcelona)

Vocales / Members:
Puri Fortes Alonso
(Centro de Investigación Médica Aplicada - CIMA/UNAV, Navarra)

Juan Francisco Lorenzo
(Hospital Universitario de Burgos)

Susanna Manrubia
(Centro de Astrobiología, CSIC-INTA, Madrid)

María Ángeles Muñoz Fernández
(Hospital General Universitario Gregorio Marañón, Madrid)

Luis Alberto Núñez Recio
(Universidad de Burgos)

Manuel Pérez Mateos
(Universidad de Burgos)

Javier Romero Cano
(Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid)

Jordi Rovira Carballido
(Universidad de Burgos)

Enrique Villar Ledesma
(Universidad de Salamanca)

JUNTA DIRECTIVA DE LA SEV / SEV EXECUTIVE BOARD

Presidente / Chairman:
Esteban Domingo Solans
(Centro de Biología Molecular "Severo Ochoa", CSIC-UAM, Madrid)

Vicepresidente / Vice Chairman:
Ricardo Flores Pedauyé
(Instituto de Biología Molecular y Celular de Plantas, CSIC-UPV, Valencia)

Secretario / Secretary:
Antonio Talavera Díaz
(Centro de Biología Molecular "Severo Ochoa", CSIC-UAM, Madrid)

Vicesecretario / Vice Secretary:
Fernando de Ory Manchón
(Centro Nacional de Microbiología, ISCIII Madrid)

Tesorero / Treasurer:
Josep Quer Sivila
(Hospital Universitari Vall d'Hebron, Barcelona)

Vocales / Members:
Rafael Blasco Lozano
(Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid)

Albert Bosch Navarro
(Universidad de Barcelona, Barcelona)

Ana María Doménech Gómez
(Universidad Complutense de Madrid, Madrid)

Rafael Fernández Muñoz
(Hospital Ramón y Cajal, Madrid)

Juan García Costa
(Complejo Hospitalario Cristal Piñor, Ourense)

Dolores García Villalón
(Secretaría Técnica de la SEV)

Esperanza Gómez-Lucía y Duato
(Universidad Complutense de Madrid, Madrid)

Mª Ángeles Muñoz Fernández
(Hospital General Universitario Gregorio Marañón, Madrid)

Amelia Nieto Martín
(Centro Nacional de Biotecnología, CSIC, Madrid)

Pilar Pérez Breña
(Centro Nacional de Microbiología, ISCIII, Madrid)

Fernando Rodríguez González
(Centre de Recerca en Sanitat Animal, UAB-IRTA, Barcelona)

Javier Romero Cano
(Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid)

Luis Valenciano Clavel
(Fundación Salud 2000, Madrid)

Enrique Villar Ledesma
(Universidad de Salamanca)

COMITÉ CIENTÍFICO / SCIENTIFIC COMMITTEE

Antonio Alcamí
(Centro de Biologia Molecular "Severo Ochoa", Madrid)

José Alcamí
(Centro Nacional de Microbiología, Madrid)

Ramón Alemany
(Institut Català d´Oncologia, Barcelona)

José María Almendral
(Centro de Biología Molecular "Severo Ochoa", Madrid)

Alfredo Berzal Herranz
(Instituto de Parasitología y Biomedicina "López Neyra", Granada)

Julià Blanco
(Hospital Universitari Germans Trias i Pujol, Barcelona)

Albert Bosch
(Universidad de Barcelona, Barcelona)

Carlos Briones
(Centro de Astrobiología, Madrid; CIBER de enfermedades hepáticas y digestivas, CIBERehd)

Javier Buesa
(Universidad de Valencia, Valencia)

María Buti
(Hospital Universitari Vall d'Hebron, Barcelona; CIBER de enfermedades hepáticas y digestivas, CIBERehd)

Joaquín Castilla
(CICbioGUNE, Bizkaia)

José Antonio Daròs
(Instituto de Biología Molecular y Celular de Plantas, Valencia)

Juana Díez
(Universidad Pompeu Fabra, Barcelona)

Ana María Doménech
(Universidad Complutense de Madrid, Madrid)

Ángela Domínguez
(Universidad de Barcelona, Barcelona; CIBER de epidemiología y salud pública, CIBEResp)

Santiago F. Elena
(Instituto de Biología Molecular y Celular de Plantas, Valencia)

Luis Enjuanes
(Centro Nacional de Biotecnología, Madrid)

José A. Esté
(Hospital Universitari Germans Trias i Pujol, Barcelona)

Juan Ignacio Esteban
(Hospital Universitari Vall d'Hebron, Barcelona; CIBER de enfermedades hepáticas y digestivas, CIBERehd)

Vicenç Falcó
(Hospital Universitari Vall d'Hebron, Barcelona)

Ricardo Flores
(Instituto de Biología Molecular y Celular de Plantas, Valencia)

Puri Fortes
(Centro de Investigación Médica Aplicada - CIMA/UNAV, Navarra)

Alberto Fraile Ramos
(Universidad Complutense de Madrid, Madrid)

Felipe García
(Hospital Clínic, Barcelona)

Juan Antonio García
(Centro Nacional de Biotecnologia, Madrid)

Fernando García-Arenal
(Universidad Politécnica de Madrid, Madrid)

Ramón García Escudero
(CIEMAT, Madrid)

Mauricio García Mateu
(Centro de Biología Molecular "Severo Ochoa", Madrid)

Juan Pablo García Muñoz
(Hospital Universitario de Burgos, Burgos)

Concepción Gimeno
(Hospital General Universitario de Valencia, Universidad de Valencia, Valencia)

Jordi Gómez
(Instituto de Parasitología y Biomedicina "López Neyra", Granada; CIBER de enfermedades hepáticas y digestivas, CIBERehd)

Susana Guix
(Universidad de Barcelona, Barcelona)

Ester Lázaro
(Centro de Astrobiología, Madrid)

Cecilio López Galíndez
(Centro Nacional de Microbiología, Madrid)

Juan Francisco Lorenzo
(Hospital Universitario de Burgos, Burgos)

Susanna Manrubia
(Centro de Astrobiología, Madrid)

Pilar Martín Duque
(Universidad Francisco de Vitoria, Madrid)

Miguel Ángel Martínez
(Hospital Universitari Germans Trias i Pujol, Barcelona)

Encarnación Martínez Salas
(Centro de Biología Molecular "Severo Ochoa", Madrid)

Antonio Mas
(Universidad de Castilla-La Mancha, Albacete)

José Antonio Melero
(Centro Nacional de Microbiología, Madrid)

Luis Menéndez Arias
(Centro de Biología Molecular "Severo Ochoa", Madrid)

María Ángeles Muñoz
(Hospital General Universitario Gregorio Marañón, Madrid)

Maria Luisa Navarro
(Hospital General Universitario Gregorio Marañón, Madrid)

Jesús Navas Castillo
(Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora", Málaga)

Amelia Nieto
(Centro Nacional de Biotecnologia, Madrid; CIBER de enfermedades respiratorias, CIBERes)

Luis Alberto Núñez
(Universidad de Burgos, Burgos)

Juan Ortín
(Centro Nacional de Biotecnologia, Madrid; CIBER de enfermedades respiratorias, CIBERes)

Fernando de Ory
(Centro Nacional de Microbiología, Madrid; CIBER de epidemiología y salud pública, CIBEResp)

Manuel Pérez Mateos
(Universidad de Burgos, Burgos)

Rosa María Pintó
(Universidad de Barcelona, Barcelona)

Josep Quer
(Hospital Universitari Vall d'Hebron, Barcelona; CIBER de enfermedades hepáticas y digestivas, CIBERehd)

David Reguera
(Universidad de Barcelona, Barcelona)

David Rodríguez Lázaro
(Instituto Tecnológico Agrario de Castilla y León, Valladolid)

Javier Romero
(Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid)

Jordi Rovira
(Universidad de Burgos, Burgos)

Juan Carlos Sáiz
(Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid)

Francisco Sobrino
(Centro de Biología Molecular "Severo Ochoa", Madrid)

Juan Evaristo Suárez Fernández
(Universidad de Oviedo, Asturias)

Enrique Villar
(Universidad de Salamanca, Salamanca)

XII CONGRESO NACIONAL DE
VIROLOGÍA

PROGRAMA / PROGRAMME

DOMINGO 9 DE JUNIO. Aula Magna de la Universidad de Burgos

15:00 – 16:30. Entrega de documentación

16:30 – 17:00. Inauguración

17:00 – 20:00. Sesión Plenaria I: The complex relationship between viruses and humans

L-1 (Conferencia Inaugural)
Chair: Enrique Villar

Influenza virus: from genes to disease
Adolfo García Sastre
Global Health and Emerging Pathogens Institute
Icahn School of Medicine at Mount Sinai, New York, USA

L-2 (Conferencia del Ganador del Premio "Virólogo Senior" de la SEV)
Chair: José Antonio Melero

New tools for the study of virus structure: Microscopies from atoms to cells
José L. Carrascosa
Department of Structure of Macromolecules
Centro Nacional de Biotecnología (CSIC), Madrid

L-3 (Conferencia Extraordinaria)
Chair: Ricardo Flores

Viruses and human evolution: from paleovirology to the role of viruses in shaping our genome
Luis P. Villarreal
Center for Virus Research
University of California, Irvine, USA

20:00 – 20:30. Concierto: *Música en evolución* (Grupo de Cámara Divertimento)

20:30 – 22:00. Cóctel de bienvenida

LUNES 10 DE JUNIO. Palacio de Congresos Fórum Evolución

9:00 – 11:00. Sesión Plenaria II (Sala de Conferencias) ***Virus evolution***

Chairs: Santiago F. Elena, Susanna Manrubia

L-4. How to get order to the viral universe
Dennis.H. Bamford
Institute of Biotechnology and Department of Biological Sciences
University of Helsinki, Finland
Comunicaciones orales: 0-1, 0-2, 0-3.

11:00 – 11:30. Café + Visita Pósters

11:30 – 13:30. Sesión Plenaria III (Sala de Conferencias): ***Hepatitis B and C: from basic virology to clinical practice***

Chairs: Juan Ignacio Esteban, Josep Quer

L-5. Biology of hepatitis C virus
Pablo Gastaminza
Laboratory for the Study of HCV Infection. Centro Nacional de Biotecnología (CSIC), Madrid.

L-6. New HCV direct acting antivirals: Key virological factors for consideration to achieve cure
Isabel Nájera
Clinical Virology, Hoffmann La Roche, Nutley, New Jersey, USA

L-7. Viral and host factors predictive of anti-HCV response
Juan Ignacio Esteban [1,2,3]
(1) Hospital Univ. Vall d'Hebron, Barcelona
(2) Universitat Autònoma de Barcelona
(3) Centro de Investigación en Red de enfermedades hepáticas y digestivas (CIBERehd), Spain

L-8. Current treatment of chronic HBV infection
Javier García Samaniego [1,2]
(1) Hospital Carlos III, Madrid
(2) Centro de Investigación en Red de enfermedades hepáticas y digestivas (CIBERehd), Spain

L-9. Anti-HCV and anti-HBV treatment in liver transplanted patients
Xavier Forns [1,2]
(1) Servicio de Hepatología. IDIBAPS. Centro Esther Koplowitz. Hospital Clinic de Barcelona
(2) Centro de Investigación en Red de enfermedades hepáticas y digestivas (CIBERehd), Spain

13:30 – 15:00. Comida

15:00 – 17:00. Sesiones Paralelas I-III

Sesión Paralela I (Sala Roja)**:** ***Immune response and vaccines***
Chairs: José Alcamí, Felipe García
Comunicaciones orales: 0-10 – 0-17.

Sesión Paralela II (Sala Verde)**:** ***Functional RNA sequences and structures***
Chairs: Juan Antonio García Álvarez, Encarnación Martínez-Salas
Comunicaciones orales: 0-18 – 0-25.

Sesión Paralela III (Sala de Conferencias): ***Epidemiology and control of viral diseases***
Chairs: Ángela Domínguez García, Fernando de Ory
Comunicaciones orales: 0-26 – 0-35.

17:00 – 17:30. Café + Visita Pósters

17:30 – 19:00. Sesiones Paralelas IV-VI

Sesión Paralela IV (Sala de Conferencias): ***Virus evolution***
Chairs: Cecilio López-Galíndez, Jesús Navas
Comunicaciones orales: 0-36 – 0-41.

Sesión Paralela V (Sala Roja): ***Hepatitis viruses***
Chairs: Antonio Mas, Rosa M. Pintó
Comunicaciones orales: 0-42 – 0-47.

Sesión Paralela VI (Sala Verde): ***Virus entry and exit, and mechanisms of viral cell-to-cell transmission***
Chairs: José María Almendral, Julià Blanco
Comunicaciones orales: 0-48 – 0-54.

19:00 – 21:00. Asamblea General SEV (Sala de Conferencias) **/ Sesión de Pósters**

MARTES 11 DE JUNIO. Palacio de Congresos Fórum Evolución

9:00 – 11:00. Sesión Plenaria IV (Sala de Conferencias): ***Virus-host interactions and genome-wide association studies***

Chairs: José Esté, Juan Ortín

L-10. Virus-host interaction at the genome level
Amalio Telenti
University Hospital, University of Lausanne, Switzerland
Comunicaciones orales: O-4, O-5, O-6.

11:00 – 11:30. Café + Visita Pósters

11:30 – 13:30. Sesión Plenaria V (Sala de Conferencias): ***New trends in virus detection in food, environmental and clinical samples***

L-11. Rapid detection of viruses in food - Are we there yet?
Chairs: Albert Bosch, Jordi Rovira
Sabah Bidawid
Health Canada, Food Directorate, Microbiology Research Division. Ottawa, Ontario, Canada
Comunicaciones orales: O-7, O-8, O-9.

13:30 – 17:30. Comida

Visita a los Yacimientos de Atapuerca
Charla introductoria:
José María Bermúdez de Castro
Co-Director de las Excavaciones de la Sierra de Atapuerca
Centro Nacional de Investigación sobre Evolución Humana, Burgos

17:30 – 19:30. Sesiones Paralelas VII-IX

Sesión Paralela VII (Sala de Conferencias): ***Virus-host interactions and genome-wide association studies***
Chairs: Juana Díez, Fernando García Arenal
Comunicaciones orales: O-55 – O-62.

Sesión Paralela VIII (Sala Verde): ***Emerging and veterinary viruses***
Chairs: Ana Doménech, Juan Carlos Sáiz
Comunicaciones orales: O-63 – O-70.

Sesión Paralela IX (Sala Roja): ***Viral replication***
Chairs: Luis Enjuanes, Luis Menéndez Arias
Comunicaciones orales: O-71 – O-78.

19:45 – 21:15. **Sesiones Paralelas X-XII**

Sesión Paralela X (Sala Verde): ***Biophysics of viruses and nanovirology***
Chairs: Mauricio García Mateu, David Reguera
Comunicaciones orales: O-79 – O-86.

Sesión Paralela XI (Sala Roja): ***HIV and other human retroviruses***
Chairs: Juan Francisco Lorenzo, María Ángeles Muñoz
Comunicaciones orales: O-87 – O-93.

Sesión Paralela XII (Sala de Conferencias): ***Plant viruses***
Chairs: José Antonio Daròs, Javier Romero
Comunicaciones orales: O-94 – O-99.

21:45 – 23:30. **Cena del Congreso**

MIÉRCOLES 12 DE JUNIO. Palacio de Congresos Fórum Evolución

9:00 – 12:00. **Sesión Plenaria VI** (Sala de Conferencias): ***Microbiology of extreme environments***
(Joint Session of the Spanish Society for Virology and the Spanish Society for Microbiology)
Chairs: Esteban Domingo, Ricard Guerrero

L-12. Life in extreme acidic habitats
Ricardo Amils [1,2]
(1) Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Madrid
(2) Centro de Astrobiología (CSIC-INTA), Torrejón de Ardoz, Madrid

L-13. Culture-independent analysis of viral assemblages from hypersaline environments
Josefa Antón
Departamento de Fisiología, Genética y Microbiología. Universidad de Alicante

L-14. Viruses in the cryosphere
Alexandre M. Anesio*, Christopher Bellas
Bristol Glaciology Centre, School of Geographical Sciences, University of Bristol, UK

L-15. Viruses from high temperature acidic environments
Kenneth Stedman[1,2]
(1) Center for Life in Extreme Environments, Portland State University, Oregon, USA
(2) Virus Focus Group, NASA Astrobiology Institute

12:00 – 12:30. Café

12:30 – 13:15. Conferencia de Clausura (Sala de Conferencias)
L-16. (Conferencia de la Ganadora del Premio "Virólogo Joven" de la SEV)
Chair: Joaquín Castilla

The chemokine-mediated immune response to viral hemorrhagic septicemia virus (VHSV) infection and vaccination in teleost fish
Carolina Tafalla
Centro de Investigación en Sanidad Animal (CISA-INIA), Valdeolmos, Madrid

13:15 – 13:45. Ceremonia de Clausura (Sala de Conferencias)
Chairs: Esteban Domingo, Puri Fortes

Entrega de premios
Presentación del XIII Congreso Nacional de Virología
Clausura

16:30 – 19:00. Visita al Museo de la Evolución Humana

19:00 – 21:30. Sesión de divulgación científica (Sala de Conferencias): ***Los virus, héroes y villanos***
Moderador: Carlos Briones

Conferencia: **Gripe, SARS, Nipah... ¿Estamos preparados para la próxima pandemia?**
Adolfo García Sastre
Global Health and Emerging Pathogens Institute
Icahn School of Medicine at Mount Sinai, New York, USA

Conferencia: **Los virus y la evolución humana: desde nuestros orígenes hasta antes de ayer**
Arcadi Navarro i Cuartiellas
Universitat Pompeu Fabra, Barcelona

Presentación de **Virópolis**, juego educativo sobre virología
Ana Doménech y Esperanza Gómez-Lucía
Universidad Complutense de Madrid

Mesa Redonda

ÍNDICE / TABLE OF CONTENTS

SESIÓN PLENARIA I

The complex relationship between viruses and humans

CHAIRS:
Enrique Villar
José Antonio Melero
Ricardo Flores

L-1 (Conferencia Inaugural)

Influenza virus: from genes to disease

Adolfo García-Sastre[1]

(1) Department of Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New York, USA.

Influenza A viruses are zoonotic pathogens that continuously circulate and change in several animal hosts, including birds, pigs, horses and humans. The emergence of novel virus strains that are capable of causing human epidemics or pandemics is a serious possibility. While the 1918 H1N1 influenza pandemic caused a devastating number of human deaths, the 2009 H1N1 pandemic was relatively mild. We have used reverse genetics to reconstruct these two pandemic viruses and study their phenotype. Virulence of the 1918 virus was multigenic, with contributions of the PB1-F2, HA, NA and NS1 genes. The antigenic similarities between the 2009 HA and HAs from human circulating viruses before 1950 explain the low incidence of infections in the elderly reported for the 2009 virus. We have investigated the potential of the 2009 H1N1 viruses to drift antigenically by acquisition of HA glycosylation sites. This was been done by generating and characterizing recombinant viruses expressing HAs with increased numbers of glycosylation sites. HA glycosylation allows escaping pre-existing immunity but reduces the ability of the virus to replicate to high titers in lungs and to cause severe disease. We also have investigated the mechanisms by how the NS1 protein of influenza virus inhibits the induction of innate antiviral responses by targeting host factors involved in the activation of the IFN system. Surprisingly, the NS1 protein can target two different host E3 ligases, both of them involved in activation of the cellular sensor RIG-I. The use of multiple mechanisms to hamper the IFN response may play a role in the ability of influenza viruses to infect multiple hosts. This zoonotic potential of influenza virus can only be successfully controlled by the generation of influenza vaccines that protect from antigenically diverse viruses present in the animal reservoir. We are presently exploring an approach for the generation of such a vaccine based on the use of immunogens that elicit a humoral response against the conserved HA stalk domain of the virus. Results in mice demonstrate that vaccination with chimeric HAs containing different HA heads, but identical HA stalks, focus the antibody response in the HA stalk, resulting in broad cross-protection. These results support the need for future clinical studies in humans to demonstrate the vaccine properties of chimeric HA constructs.

Keywords: influenza, immunity, vaccines, pathogenesis.

L-2 (Conferencia del Ganador del Premio "Virólogo Senior" de la SEV)

New tools for the study of virus structure: microscopies from atoms to cells.

José L. Carrascosa[1]

(1) Department of Structure of Macromolecules, Centro Nacional de Biotecnología (CSIC), Madrid.

The development during recent years of cryo-electron microscopy methods, coupled to three-dimensional reconstruction and image processing, has given rise to a powerful new tool for solving the structure of regular viruses at nanometric resolution. Several icosahedral viruses have been solved at atomic resolution using this approach [1], and many other viral related STRUCTURES HAVE BEEN SOLVED At 1-2 nm resolution, highlighting the complexity of virus maturation processes [2, 3]. To tackle the analysis of non-regular viruses, as well as to study their assembly and maturation inside the cellular environment a number of approaches have been developed, mostly related to cryo-electron tomographic reconstructions. These methods, combined with modeling and threading of atomic structures of specific structural components into the electron microscopy volumes, have revealed interesting insights of the viral cycle inside the cells [4, 5].

The need to integrate a more quantitative mapping of the cell components in a comprehensive integral view combining structure, proteomics and interactions, among other "omics", is driving different methodological attempts. One of them is X-ray microscopy, which overcomes the penetration limit of classical electron microscopy. Cryo-X-ray tomographic reconstructions of whole cells have revealed exciting perspectives to detect viral structures inside whole cells without the need of any fixation or staining, thus opening the way to quantitative correlative analysis of virus-cell interactions at resolutions around 20-30 nm [6].

[1] Yu X, Jin L, Zhou ZH (2008) 3.88. Nature 453:415-419
[2] Agirrezabala X, Martin-Benito J, Caston JR, Miranda R, Valpuesta JM, Carrascosa JL (2005). EMBO J. 24:3820-3829
[3] Ionel A, Velazquez-Muriel JA, Luque D, Cuervo A, Caston JR, Valpuesta JM, Martin-Benito J, Carrascosa JL (2011). J Biol Chem 286:234-242
[4] Grunewald K, Cyrklaff M (2006). Curr. Opin. Microbiol 9:437-442
[5] Cyrklaff M, Risco C, Fernandez JJ, Jimenez MV, Esteban M, Baumeister W, Carrascosa JL (2005). Proc Natl Acad Sci U S A 102:2772-2777
[6] Chichon FJ, Rodriguez MJ, Pereiro E, Chiappi M, Perdiguero B, Guttmann P, Werner S, Rehbein S, Schneider G, Esteban M, Carrascosa JL (2012). J Struct Biol 177:202-211

Keywords: virus structure determination, three-dimesional reconstruction, cryo-electron microscopy, X-ray cryo-tomography.

L-3 (Conferencia Extraordinaria)

Viruses and human evolution: from paleovirology to the role of viruses in shaping our genome

Luis P. Villarreal[1]

(1) Center for Virus Research, University of California, Irvine, USA.

Humans have evolved a much larger and more social brain than other primates, yet the human genome has similar gene number to that of simple animals. Human and chimpanzee DNA differ mostly due to non-coding intragenic DNA derived from retroposons and retroviruses and account for

large numbers of 'indels'. Recently, noncoding DNA was observed to be highly transcribed and appears to exert RNA mediated regulatory control. Intron residing alu's and miRNA elements, seem particularly involved in brain network regulation and have undergone recent human specific expansion. Accounting for this rapid network expansion by 'junk' DNA is daunting from a classical Darwinian perspective.

Here I outline an alternative view involving 'virolution': virus as mediators of host evolution and as agents competent to edit host code. Originally, RNA virus evolution (quasispecies, QS) was proposed to result from errors of a master fittest individual type. But an updated definition includes cooperative, consortial, quasispecies (QS-C) that retain identity of the RNA society. From this perspective, I reexamine the early (pre-DNA) evolution (e.g. ligated stem-loops, ribozymes, viroids and the ribosome) as function of an RNA society. Such social RNA's exhibits multilevel selection and can be considered as occupying a DNA habitat (paleoviruses). An early identity function for the alu containing 7S RNA can be found here. RNA consortia also promote network formation. But successful RNA colonization of a host requires strategies (addiction modules) that select for this RNA. This selection will often involve acquisition of a new antiviral capacity. I then examine human evolution from a virolution premise. Viral regulation of anti-viral signal transduction and virus mediated changes to stem cells and reproductive tissues (a virus rich species specific habitat) are emphasized. Retrovirus involvement in altering stem cell regulatory RNA is now clear. Embryo implantation via the placenta along with placental control of brain development and maternal (bound) social behavior are also used to evaluate how RNA societies affect evolution of brains and social bonds. Massive ERV involvement in the origin of the placental network is established. Humans, however, required a more invasive version of a placenta to support a large fetal brain. Viruses are competent to control all these processes and I suggest their involvement.

I also examine possible ERV-LINE mediated distributing the 7S derived alu elements and how this may result in a new RNA based regulatory and immune response. Finally, I examine recent results from the Neanderthal genome that establish the human introgression of Neanderthal antiviral genes, as well as differences in endogenous retroviruses. I present evidence that human and Neanderthals also likely differed in species specific viruses of reproductive tissue (especially herpes viruses), which likely affected breeding outcomes (via encephalitis). Finally I reconsider how viruses associated with RNA regulated reproductive tissues could have promoted the alteration of human social bonding systems.

Keywords: RNA world, quasispecies, virus evolution, paleovirology, endogenous retroviruses, virus-host coevolution, human evolution.

SESIÓN PLENARIA II

Virus evolution

CHAIRS:
Santiago F. Elena
Susanna Manrubia

L-4

How to get order to the viral universe

Dennis.H. Bamford[1]

(1) Institute of Biotechnology and Department of Biological Sciences. University of Helsinki, Finland.

Viruses are the most abundant living entities in the biosphere outnumbering their host organisms by one to two orders of magnitude. It is conceivable that they cause the highest selective pressure their hosts encounter. As obligate parasites viruses are dependent on their hosts but their origins seem to deviate from that of cellular life.

What are the possible structural principles to build viruses is an open question. However, structural studies on virus capsids and coat protein folds propose that there are only a limited number of ways to construct a virion. This limitation is based on the limited protein fold space. Consequently, relatedness of viruses is not connected to the type of cells they infect and the same architectural principle of the capsid has been observed in viruses infecting bacteria as well as humans. Using the viral capsid architecture it is possible to group viruses to several structural lineages that may have existed before the three cellular domains of life (bacteria, archaea and eukarya) were separated. This would mean that viruses are ancient and that early cells were already infected with many different types of viruses proposing that the origin of viruses is polyphyletic opposing to the monophyletic origin of cellular life. To test the hypothesis of limited viral structure space we have collected information on randomly collected environmental viruses infecting archaea in particular and compared the obtained information to known viral structures.

Keywords: virus evolution, origin of viruses, phylogeny, structure.

O-1

Infectivity decline of an RNA plant virus by increased mutagenesis supports the lethal defection model *in vivo*

Luis Díaz Martínez[1], Isabel Brichette Mieg[1], Ana Grande-Pérez*[1]

(1) Área de Genética, Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" (IHSM-UMA-CSIC). Universidad de Málaga, Consejo Superior de Investigaciones Científicas.

Lethal mutagenesis is a new antiviral therapy based on increasing the mutation rate by using mutagenic base and nucleoside analogues whose molecular mechanisms are not fully understood. Most of the research has been conducted on animal RNA viruses in cell culture and, to a lesser extent, *in vivo*. There is experimental evidence supporting the model of lethal defection for lethal mutagenesis of RNA viruses. In this model, viral genomes with a low degree of mutation and low specific infectivity, termed "defectors", exert an

interfering activity leading to virus loss. Lethal mutagenesis of plant viruses has not been addressed yet despite being excellent *in vivo* model systems that develop systemic infections, undergo rapid bottlenecks and pose no ethical issues.

Here, we address lethal mutagenesis *in vivo* of Tobacco mosaic virus (TMV), a single-stranded positive RNA virus of 6.4 Kb. *Nicotiana tabacum* plants cultured *in vitro* were treated with 25, 50 and 100 µg/ml of the base analogue 5-fluorouracil (FU) and 24 h later were inoculated with 50 lesion forming units (lfu) of TMV. We analyzed the infectivity, viral load and mutant spectra of viral populations after 5 and 10 days of treatment, as well as of populations that went 10 days of treatment followed by 21 days of *ex vitro* growth in the absence of FU.

The results show that TMV infectivity decreases when treated with 50 and 100 µg/ml FU for 10 days. TMV mutagenized populations grown without FU reach infectivity values higher than untreated populations. Predominant mutations in FU-treated populations with decreased infectivity at 10 dpi are U C, A G and G A transitions, which are expected due to the action of FU. TMV replication is not affected by FU at any dose and there are no imbalances of ribonucleotide triphosphate pools measured by HPLC. No differences in mutation frequencies and Shannon Entropies between control and FU-treated populations with declined infectivity were found. However, we did found a dose-dependent decrease of specific infectivity in FU-treated populations, but not in untreated samples, as well as dominance of molecules with a low degree of mutation. Specific infectivity recovered to control levels after 21 days of growth without the analogue. Altogether, our results suggest that TMV defector molecules mediate the decrease in TMV infectivity. This is the first report that addresses the molecular basis of lethal defection *in vivo* using an RNA plant virus.

Keywords: antiviral therapy, lethal mutagenesis, lethal defection, 5-fluorouracil, tobacco mosaic virus, specific infectivity, Nicotiana tabacum.

O-2

Novel papillomaviruses in free-ranging Iberian bats challenge the dogmas: interspecies transmission, no virus-host coevolution and evidences for recombination

Ignacio G Bravo*[1], Juan E Echevarría[2], Javier Juste[3], Gudrun Wibbelt[4], Raquel García Pérez[1]

(1) Laboratorio de Infecciones y Cáncer. Instituto Catalán de Oncología. L'Hospitalet, Barcelona(2) Centro Nacional de Microbiología. Instituto de Salud Carlos III. Majadahonda, Madrid (3) Estación Biológica de Doñana. CSIC. Sevilla (4) Patologie und Bakteriologie. Leibniz-Institut fuer Zoo- und Wildtierforschung, Berlín, Alemania.

Papillomaviridae are a wide and divergent family of small, non-encapsulated dsDNA viruses that infect virtually all mammals and possibly most of vertebrates. Papillomaviruses (PVs) are epitheliotropic and the clinical manifestations of the infections range from asymptomatic to proliferative lesions such as warts or malignant lesions that

may generate anogenital cancers. Animal PV diversity is poorly sampled, and thence most of our hypotheses about PV evolution are biased because of the clinical focus on human PV research.

We communicate here the sequencing and cloning of five new PVs isolated from different bat species: *Eidolon helvum PV type 1* (EhelPV1), *Rhinolophus ferrumequinum PV type 1* (RferPV1), *Eptesicus serotinus PV type 1* (EserPV1), *Eptesicus serotinus PV type 2* (EserPV2) and *Eptesicus serotinus PV type 3* (EserPV3). These novel PVs were isolated from individua from three different bat families: Pteropodidae, Rhinolophidae y Vespertilionidae, respectively. Further, we have studied the prevalence of EserPV1, 2 and 3 in a number of *E. serotinus* and *E. isabellinus* bat colonies in the Iberian peninsula.

Phylogenetic relationships within Papillomaviridae were inferred by means of maximum likelihood, Bayesian, and supernetwork analyses. Further, we have studied the evolution of regulatory motifs independently of phylogeny.

There are currently nine bat PVs, and they are not monophyletic: five of them belong to the Lamda+Mu PVs crowngroup, one to the Alpha+Omicron PVs crowngroup, and three of them are close to root and their precise phylogenetic positions cannot be inferred with certainty. The increased sampling of the Lambda+Mu PV crowngroup is welcome, as it hosts viruses infecting diverse hosts (carnivores, chiroptera, primates, rodents and lagomorpha), with diverse tropisms (cutaneous and mucosal) and with divergent clinical manifestations of the infections (asymptomatic, benign proliferations and malignant tumours). Our results question once again the old assumption of virus-host coevolution for the PVs and reinforce the hypothesis of a biphasic evolution in the story of the PVs: an initial adaptive radiation linked to the definition of new niches during the evolution of mammalian skin that generated the ancestral PV crowngroups, followed by a limited co-speciation between viruses and hosts.

[This work was partially funded by the disappeared Ministerio de Ciencia e Innovación, CGL2010-16713.]

Keywords: papillomavirus, virus-host coevolution.

O-3

Evolution of increased pathogenicity in a plant virus results in fitness costs affecting different life-history traits

Aurora Fraile[1], Jean-Michel Hily[1], Israel Pagan[1], Luis F. Pacios[1], Fernando García Arenal*[1]

[1] Centro de Biotecnología y Genómica de Plantas UPM-INIA. Universidad Politécnica de Madrid. Pozuelo de Alarcón, Madrid.

Despite that plant viruses are virulent crop parasites, and that resistance in crops selects for increased pathogenicity in viruses, virus evolution in crops is poorly understood, and there is no clear demonstration of plant-virus co-evolution. We

have analysed the reciprocal effects of virulence of viruses and defence of plants on plant and virus fitness, and on whether they lead to co-evolution. Our work model was based on tobamoviruses that are controlled by, or overcome, different resistance alleles at the *L* locus bred into cultivars of pepper (*Capsicum annuum*) crops according to a gene-for-gene (GFG) interaction. As predicted by models of the evolution of GFG systems, data on the long-term evolution of field tobamovirus populations suggested that evolution to increased pathogenicity in response to the selection exerted by resistance would have fitness penalties. These penalties were demonstrated in experiments in which virus multiplication of isolates belonging to different pathotypes were assayed in different host genotypes either in single infection or in competition in mixed infection. We also showed that amino acid substitutions in the virus coat protein that result in overcoming the *L*-gene resistance affect the stability of the virions and, hence, their survival out of the host cells. Thus, plant resistance selects for traits in the virus population related to plant-virus interaction, such as its increase in pathogenicity, but also for traits unrelated to the plant-virus interaction, such as altered survival. Both of those traits affect virus fitness.

Keywords: fitness trade-offs, reproduction, survival, plant-virus coevolution.

SESIÓN PLENARIA III

Hepatitis B and C: from basic virology to clinical practice

CHAIRS:
Juan Ignacio Esteban
Josep Quer

L-5

Biology of hepatitis C virus

Pablo Gastaminza[1]

(1) Laboratory for the Study of HCV Infection, Centro Nacional de Biotecnología (CSIC), Madrid.

It is estimated that more 170 million humans are infected by hepatitis C viruses (HCV). HCV establishes chronic infections in the majority of the infected individuals. These patients display chronic hepatitis and fibrosis that often leads to cirrhosis and hepatocellular carcinoma. HCV is not directly cytolytic and it is thought that immune system-mediated events are major players in HCV-related pathogenesis although chronic carriers fail to mount effective immune responses to clear the virus. There is no vaccine against HCV and the current therapy is plagued with adverse effects and expensive, although the recent inclusion of specific antiviral components significantly improved the cure rate. Hepatitis C viruses are members of the *Flaviviridae* family and have a single stranded RNA genome of positive polarity that encodes a unique open reading frame, flanked by highly structured non-coding RNA sequences that regulate viral translation and replication. The resulting polyprotein is sequentially cleaved by cellular and viral proteases to generate all the viral proteins. HCV replicates in the cytoplasm of human hepatocytes, although other cell types are suspected to be susceptible to HCV infection. HCV virions are enveloped and display a characteristic low buoyant density that is part due to their association with components of host lipoproteins. This physical association illustrates the tight functional links between cellular lipid homeostasis and HCV replication. In fact, chronic HCV infection causes abnormal lipid accumulation in the liver parenchyma (steatosis), which is observed in around 50% of the patients and is a direct consequence of the virus-induced deregulation of lipid homeostasis. HCV infection persists both in patients and cell culture for long periods of time by subtly modifying normal cellular metabolism, including blunting innate immune responses, remodeling intracellular membranes and co-opting important biosynthetic pathways of the host cell. In this lecture, we will discuss the current knowledge of basic aspects of HCV infection and the current state-of-the-art tools for the study of HCV infection in cell culture.

Keywords: HCV, structure, replication, function, persistence, infection.

L-6

New HCV direct acting antivirals: Key virological factors for consideration to achieve cure

Isabel Nájera[1]

(1) Clinical Virology, Hoffmann La Roche, Nutley, New Jersey, USA.

Two inhibitors of the NS3/4A serine protease are now approved for clinical use (telaprevir and bo-

ceprevir) that achieve sustained-virological response rates of ~70% in genotype 1. However, resistance develops quickly even when these drugs are administered in combination with peginterferon/ribavirin. A number of direct acting antivirals (DAAs) are being evaluated in clinical trials, targeted primarily against the hepatitis C (HCV) NS3-4A protease, NS5A and NS5B polymerase. DAAs have been shown to be potent inhibitors of HCV RNA replication and key for the cure of HCV infection. Recent data have shown the clear need for combination therapy either with Pegylated Interferon and Ribavirin or in Interferon free regimens, with or without ribavirin, in order to achieve high levels of cure of HCV infection. A number of virological factors, tightly related to HCV intrinsic variability, have been described to affect therapeutic efficacy: i) drug susceptibility of different genotypes and subtypes, ii) prevalence of known DAA-resistant virus among untreated patients, iii) ease of drug resistance selection while on treatment, and iv) the genetic barrier to resistance, that can be related to the virus genotype or subtype the patient is infected with. I will present and discuss these main virological factors and how they can affect treatment efficacy to be taken into consideration for the development of combination therapy approaches.

Keywords: HCV, antivirals, DAAs, drug resistance, genetic barrier, subtype.

L-7

Viral and host factors predictive of anti-HCV response

Juan Ignacio Esteban[1,2,3]

(1) Hospital Univ. Vall d'Hebron, Barcelona. (2) Universitat Autònoma de Barcelona. (3) Centro de Investigación en Red de enfermedades hepáticas y digestivas (CIBERehd).

Persistent infection of Hepatitis C Virus (HCV) is the leading cause of chronic hepatitis and cirrhosis, is responsible of 60% of hepatocellular carcinoma (HCC) and over 50% of liver transplants (TxH) between adults in our geographic area. The emergence of new direct-acting inhibitors (DAAs) used in combination with standard therapy (PegIFN+RBV) increases the effectiveness of sustained virologic response (SVR) which is associated with healing. At present more than 60 new DAAs are in different clinical or pre-clinical studies. The problem with these new treatments, is, besides the high cost, the increase in some instances severely, the substantial side effects caused by peg IFN + ribavirin. From this comes the need to improve the prediction of response / non-response in order to guide more effective personalized treatment and avoid unnecessary side effects. The baseline predictors of SVR include both viral factors and patient. Factors include viral, viral load, quasispecies complexity, the presence of resistant mutants in the pretreatment correct classification into subtypes and detecting multiple concurrent infections or recombinations. Regarding patient factors besides personal factors such as age, weight, sex, ethnicity, and disease as ALT, fibrosis,

obesity, fatty liver, etc.; Described polymorphisms in genomic DNA directly associated with SVR or complications treatment: IL28; ITPA; hCNT2 RBV receiver, ISGs (OASL and IFIT1) HLAC-C; KIR, among others. Define more efficient predictors and study pharmacokinetic interactions have become a necessity.

Keywords: HCV, antiviral response, DAAs, quasispecies, variability, polymorphisms.

L-8

Current treatment of chronic HBV infection

Javier García Samaniego*[1,2]

(1) Hospital Carlos III, Madrid. (2) Centro de Investigación en Red de enfermedades hepáticas y digestivas (CIBERehd).

Chronic hepatitis B virus (HBV) infection is a potentially serious disease that can lead to cirrhosis, end-stage liver disease, and hepatocellular carcinoma (HCC). It is estimated that 400 million people worldwide are chronically infected with HBV. The primary goal of therapy for chronic hepatitis B is long-term suppression of serum HBV DNA, which will likely decrease progression to cirrhosis and HCC. However, chronic HBV infection cannot be completely eradicated due to the persistence of covalently closed circular DNA (cccDNA) in the nucleus of infected hepatocytes, which may explain HBV reactivation. The indications for treatment are generally the same for both HBeAg-positive and HBeAg-negative patients. This is based mainly on the combination of three criteria: serum HBV DNA levels, serum ALT levels and severity of liver disease. Patients should be considered for treatment when they have HBV DNA levels above 2000 IU/ml, serum ALT levels above the upper limit of normal and severity of liver disease assessed by liver biopsy (or non-invasive markers such as transient elastometry) showing moderate to severe active necroinflammation and/or at least moderate fibrosis using a standardized scoring system.

The medications currently approved for the treatment of chronic hepatitis B include interferon alfa-2b, peginterferon alfa-2a (PEG-IFN), and the oral nucleoside/nucleotide analogs (NA) lamivudine, adefovir dipivoxil, entecavir, telbivudine and tenofovir. Each of these drugs has a number of advantages and disadvantages. Issues for consideration in the selection of therapy include efficacy, safety, incidence of resistance, method of administration, and cost. Currently, there are two different treatment strategies for both HBeAg-positive and HBeAg-negative patients: treatment of finite duration with PEG-IFN or a NA and long-term treatment with NA. The main theoretical advantages of PEG-IFN are the absence of resistance and the potential for immune-mediated control of HBV infection with an opportunity to obtain a sustained virological response off-treatment and a chance of HBsAg loss in patients who achieve and maintain undetectable HBV DNA. Frequent side effects and subcutaneous injection are the main disadvantages of PEG-IFN treatment. PEG-IFN is contraindicated in patients with decompensated HBV-related cirrhosis or autoimmune disease, in patients with uncontrolled severe depression or psychosis, and in female patients during pregnancy. Entecavir and tenofovir are potent HBV inhibitors with a high barrier to resist-

ance. They can be confidently used as first-line monotherapies whereas the other three NAs should only be used in the treatment of chronic hepatitis B if more potent drugs with high barrier to resistance are not available.

Keywords: HBV, persistence, antivirals, treatment, resistance, genetic barrier.

L-9

Anti-HCV and anti-HBV treatment in liver transplanted patients

Xavier Forns[1,2]

(1) Servicio de Hepatología. IDIBAPS. Centro Esther Koplowitz. Hospital Clinic de Barcelona. (2) Centro de Investigación en Red de enfermedades hepáticas y digestivas (CIBERehd).

Liver transplantation (LTx) is the only alternative for patients with end-stage liver cirrhosis or hepatocellular carcinoma. Hepatitis B and C (particularly C) are the most common cause of cirrhosis and liver cancer in our environment and therefore the first indication of LTx. Since the introduction of anti-HBV immunoglobulin and especially new oral antivirals, the recurrence of post-transplant HBV is no longer a problem and patients have an excellent prognosis. Conversely, post-transplant HCV is the leading cause of liver graft loss in the long term and represents a real problem for the majority of transplant programs. Among the strategies we have to prevent this infection in the graft include antiviral treatment on the waiting list (only in patients with compensated cirrhosis) and treatment in the post-transplant (when liver damage has occurred). These strategies achieve HCV cure infection in one third of patients. However, it is likely that in the next years prevention or cure of HCV infection will be achieved in a much larger proportion of cases using direct antiviral agents (DAAs) that provide greater efficiency, tolerance and safety.

Keywords: HCV, HBV, liver transplantation, treatment, DAAs.

SESIÓN PLENARIA IV

Virus-host interactions and genome-wide association studies

CHAIRS:
José Esté
Juan Ortín

L-10

Virus-host interaction at the genome level

Amalio Telenti[1]

(1). University Hospital, University of Lausanne, Switzerland.

In the field of genomics, the interaction host-pathogen can be investigated under the prism of genome-to-genome interactions. The interaction can be seen in the frame of long-term evolutionary conflicts (eg. lentiviruses and primates), intraspecies pressures (eg. genome-wide association analysis of host-virus genetic variants), and in a dynamic mode (eg. genetic reprogramming of the cell during viral invasion).

Three topics will be presented to illustrate these concepts (i) the screening of innate immunity effectors using evolutionary genomics, (ii) the mapping of host pressures on HIV, genome-wide, and (iii) the modeling of the replication cycle in the infected cell.

1. Paradigmatic antiretroviral genes (TRIM5a, APOBE3G, BST2, SAMHD1) are under positive selective pressure in primates, interferon dependent, and interact with viral proteins. We used this signature to screen the human genome: of 21389 genes, 841 (4%) were identified because of features of positive selection in primates. Among those genes under positive selective pressure, only 30 genes/proteins (0.14%) were identified through orthogonal datasets as being upregulated during HIV infection *in vivo*, and/or plausibly interacting with HIV proteins. When overexpressed, several genes associate with a profound reduction of HIV replication in the cotransfected cell.

2. Host and pathogen genetic variation can be explored by joint analysis of both genomes. To this aim, whole genome sequences from HIV, paired with genome-wide genotyping of the corresponding host, was obtained for over 1000 individuals. These resulted in mapping of sites on the viral genome that are associated with human variants.

3. The analysis of host-HIV interaction at the cellular level was achieved by repeated measurements in a standardized cell system and mathematical modeling. It allowed the association between the emergence of viral replication intermediates and their impact on the cellular transcriptional response with high temporal resolution. 73% of host expressed genes are modulated in concordance with key steps of viral replication. This profound perturbation of cellular physiology was investigated in the light of several regulatory mechanisms, including transcription factors, miRNAs, host-pathogen interaction, and proviral integration.

These three genomics approaches interrogate the nature of the mutual influences at the long-term evolutionary scale, at the human population scale, and at the cellular level.

Keywords: evolutionary genomics, GWAS, transcription; HIV.

0-4

Secreted herpes simplex virus-2 glycoprotein G modifies NGF-TrkA signalling to attract free nerve endings to the site of infection

Jorge R. Cabrera*[1], Abel Viejo Borbolla[2], Nadia Martínez Martín[2], Francisco Wandosell[1,4], Antonio Alcamí[2,3]

(1) Departamento de Neurobiología Molecular. Centro de Biología Molecular Severo Ochoa, Consejo Superior de Investigaciones Científicas, Universidad Autónoma de Madrid. (2) Departamento de Virología y Microbiología. Centro de Biología Molecular Severo Ochoa, Consejo Superior de Investigaciones Científicas, Universidad Autónoma de Madrid. (3) Department of Medicine. University of Cambridge, United Kingdom (4) Centro de Investigaciones Biológicas en Red de Enfermedades Neurodegenerativas (CIBERNED).

Herpes simplex virus type 1 and 2 (HSV-1 and HSV-2, respectively) are neurotropic viruses that infect neurons of the peripheral nervous system (PNS), establishing latency. The neurotropism of HSV is crucial for pathogenesis and constitutes a major evolutionary advantage due to the particularities of the immune system within the nervous system. The molecular mechanisms facilitating the infection of peripheral neurons have not been fully dissected. Neurotrophic factors are secreted proteins that play relevant roles in neuronal survival, axonal growth and guidance in the PNS. Here we show that the viral chemokine-binding protein glycoprotein G from HSV-1 and HSV-2 interact with several neurotrophic factors, including nerve growth factor (NGF). Binding of secreted glycoprotein G from HSV-2 (SgG2) to NGF increases NGF-dependent axonal growth of sympathetic neurons *ex vivo*. SgG2 modifies the signalling pathways downstream of the NGF receptor, TrkA, changing TrkA relationship with the neurotrophin receptor p75. SgG2 alters TrkA recruitment to lipid rafts, decreases TrkA internalization and partially blocks TrkA retrograde transport. *in vivo*, both HSV-2 infection and SgG2 expression in mouse hindpaw epidermis enhances axonal growth modifying the termination zone ofthe NGF-dependent peptidergic free nerve endings. This constitutes the discovery of the first viral protein with the ability to bind to and modulate neurotrophic factors, which may facilitate HSV-2 access to peptidergic free nerve endings in the epidermis and invasion of the nervous system. This dual function of the chemokine-binding protein SgG2 uncovers a novel strategy evolved by HSV-2 to modulate factors from both the immune and nervous systems.

Keywords: herpes simplex virus, neurotropic viruses, neurotrophic factors, NGF, TrkA.

0-5

Potyviral P1 protein traffics to the nucleolus, associates with the host 60S ribosomal subunits and stimulates viral translation

Fernando Martínez[1], José Antonio Daròs*[1]

(1) Instituto de Biología Molecular y Celular de Plantas. CSIC-Universidad Politécnica de Valencia.

Genus Potyvirus gathers approximately 30% of known plant viruses, many of them causing important problems in agriculture. Potyviral genome is an about 10000 nt long RNA molecule of positive sense that encodes a large polyprotein processed by three viral proteinases. In contrast to host mRNAs, potyviral genomic RNA contains a viral protein genome-linked (VPg) at the 5' end and a 5' untranslated region with internal ribosome entry site (IRES) activity which facilitate its translation in host infected cells. P1 proteinase, the most amino-terminal product of the polyprotein, is an accessory factor stimulating viral genome amplification whose role during infection is mostly unknown. In our work, we infected plants with Tobacco etch virus (TEV; genus Potyvirus) recombinant clones in which P1 was tagged with a fluorescent protein to track its expression and subcellular localization or with an affinity tag to identify proteins forming complexes with P1 during infection. The fluorescent-tagged P1 was tracked during infection by confocal laser scanning microscopy. The host proteins interacting with P1 during infection were purified by chromatography and identified by mass spectrometry analysis. Our results showed that TEV P1 exclusively accumulates in infected cells at an early stage of infection, and that the protein displays a dynamic subcellular localization trafficking in and out of the nuclei during infection. Inside the nucleus, P1 particularly targets the granular component of nucleolus, where assembly of ribosomal particles takes place. Consistently, we found functional nucleolar localization and nuclear export signals in TEV P1 sequence. Our results also indicated that TEV P1 physically interacts with the host translational machinery and specifically binds to the 60S ribosomal subunits during infection. Furthermore, *in vitro* translation assays of reporter proteins in bicistronic constructs showed that TEV P1 stimulates protein translation, particularly when driven from the TEV internal ribosome entry site. These assays also showed that TEV helper-component proteinase (HC-Pro) inhibits protein translation. These findings constitute the first evidence that potyviral P1 participates in viral translation. We propose that the coordinated action of potyviral proteins P1 and HC-Pro subvert the host translation machinery to ensuring synthesis of viral proteins in infected cells, stimulating viral translation and inhibiting host translation, respectively.

Keywords: RNA virus, plant virus, potyvirus, host plant-virus interaction, translation regulation, nucleolar localization.

O-6

A SARS-cov lacking e gene induced reduced levels of inflammation, mediated by a limited NF-KB activation

Marta L deDiego[(1)], José L Nieto-Torres[(1)], José M Jiménez Guardeño[(1)], José A. Regla Nava[(1)], Raúl Fernández Delgado[(1)], Luis Enjuanes*[(1)]

(1) Department of Molecular and cell Biology. National Center of Biotechnology (CNB-CSIC), Madrid.

Deletion of the structural envelope E gene from a severe acute respiratory syndrome coronavirus

(SARS-CoV) led to an attenuated virus (SARS-CoV-deltaE). To analyze the mechanism of attenuation, differential gene expression in cells infected by viruses with and without E protein was compared. Stress response and unfolded protein response genes were upregulated in cells infected by SARS-CoV-deltaE compared to the infection by SARS-CoV with E protein. The addition of E protein in trans reduced the stress response induced in SARS-CoV-deltaE and RSV-infected cells, and in cells treated with different drugs, indicating that this effect was specific. The expression of proinflammatory cytokines was reduced in lungs of mice infected with a mouse adapted SARS-CoV-MA15-deltaE compared to lungs infected with the mouse adapted virus including E protein. The induction of virus induced inflammatory responses may be mediated by the activation of five major signaling pathways (IRF3/7, ATF-2/Jun, AP-1, NF-KB, and NF-AT), but in infections by SARS-CoV with and without E protein the only pathway differentially activated was NF-KB. Interestingly, the addition of an specific inhibitor of NF-KB led to a reduced inflammatory response after SARS-CoV infection, confirming that NF-KB mediates the induction of inflammation after SARS-CoV infection. A reduction in neutrophil migration to sites of lung inflammation was observed in mice infected with SARS-CoV-MA15-deltaE, what probably contributed to the lower degree of inflammation detected and to SARS-CoV-deltaE attenuation. The mutant SARS-CoV missing E protein provided protection against challenge with homologous and heterologous pathogenic SARS-CoVs in hamsters and transgenic mice. Furthermore, the mouse adapted SARS-CoV-MA15-deltaE provided complete long-term protection against a virulent mouse adapted SARS-CoV in both young and old Balb/c mice, indicating that SARS-CoV-deltaE is a promising vaccine candidate.

Keywords: SARS-CoV, envelope protein, virus attenuation, stress response, NF-KB, inflammation, vaccine.

SESIÓN PLENARIA V

New trends in virus detection in food, environmental and clinical samples

CHAIRS:
Albert Bosch
Jordi Rovira

L-11

Rapid detection of viruses in food- are we there yet?

Sabah Bidawid[1]

(1) Health Canada, Food Directorate, Microbiology Research Division, Ottawa, Ontario, Canada.

There's no denying of the fact that outbreaks due to foodborne viruses are on the rise world-wide. Like-wise, our capacity to detect, identify and characterise viral agents responsible for these outbreaks has also improved. These improvements are evident in the adaptation, modification and development of more rapid virus detection technologies. Nevertheless, irrespective of how fast and efficient the detection method is, its performance is essentially governed by how fast and efficient is the process of virus recovery from complex food matrices. Major challenges in this recovery process remain largely unresolved due to a number of well recognized factors such as 1) the melange of complexity and variation of food matrices, 2) the low number of viruses present in food, 3) interference of inhibitors present in foods with the efficiency and performance of virus isolation and molecular detection. Furthermore, our inability to lab-culture certain key foodborne viruses, such as norovirus, has greatly hindered our ability to establish homogenous workable stock cultures of these viruses for experimental designs and method development.

All in all, the development of more rapid and sensitive methods to detect isolated virus is moving at a much faster pace than our ability to rapidly and efficiently isolate the virus itself from the food it contaminates. Advances in virus detection methodologies are well established in such technologies as the conventional reverse transcriptase polymerase chain reaction (PCR), real-time PCR, Microarray Chip technologies, Sequence-based analysis, and more recently, biosensors, lab-on-a chip microfluidics-based detection, and the outbreak-predictive remote sensing technology. On the other hand, progress in developing more rapid, efficient, and less labour-intensive techniques for virus isolation from food complex matrices is moving at a considerably slower pace. Nevertheless, although we still rely on two basic isolation schemes; adsorption-elution-concentration and extraction-concentration methods; certain recent advancements have been introduced such as immunomagnetic bead separation, filtration and rapid multi-stage sample preparation.

This presentation will provide an overview of current and recent advancements in foodborne virus isolation and concentration methods, viral nucleic acid extraction from food matrices, and molecular-based detection and characterisation approaches.

Keywords: foodborne viruses, rapid detection, novel technologies.

O-7

Food safety in Europe: improvement and cost reduction of standard methods

Susana Guix*(1), Cristina Fuentes(1), Francisco Pérez Rodríguez(1), Rosa M. Pintó(1), Albert Bosch(1)

(1) Department. of Microbiology. University of Barcelona

Viruses such as norovirus (NoV) and hepatitis A virus (HAV) are a worldwide leading cause of food borne illnesses and outbreaks. Contamination of food products with these viruses can occur at any step in the food production chain, for example at pre-harvest stages, during harvest, and at the post-harvesting stages (processing or preparation), and the number of reported outbreaks following these patterns has recently increased. Nucleic acid amplification techniques are currently the most widely used methods for detection of viruses in food, and (RT-) PCR and q(RT-) PCR remain the current gold standard methods. However, the inclusion of virus analysis in regulatory standards for viruses in food or water samples must overcome several shortcomings, among others, the technical difficulties and high costs of virus monitoring, the lack of harmonized and standardized assays and the challenge posed by the ever changing nature of the principal target viruses. Molecular procedures must be simplified and automated before they could be adopted by routine monitoring laboratories and considered by regulatory agencies when formulating guidelines for virus standards.

In 2004 the European Committee on Standardization (CEN) tasked a technical advisory group (CEN/TC275/WG6/TAG4) with the development of a standard method for detection of NoV and HAV in selected foodstuffs. The CEN / TC275/WG6 / TAG4 committee developed ISO proposals for sensitive and quantitative qRT-PCR based methods for the detection of HAV and NoV that will enable the formulation of regulatory standards for virus in food. In this context, our laboratory has also developed a multiplex real-time qRT-PCR assay for the simultaneous detection of HAV, NoV GI, NoV GII and Mengovirus (used as process control for determination of the virus/nucleic acid extraction efficiency). Serial dilutions of viral RNAs and plasmid targets were used to compare standard curves of multiplex and monoplex qRT-PCR assays for the all four viruses. Additionally, an overall good concordance between both qRT-PCR methods was also observed when performed on naturally contaminated shellfish and water samples.

The multiplex assay herein reported is similar to the corresponding monoplex assays in terms of sensitivity and specificity, being a time-saving and cost-effective tool to detect and accurately quantify HAV, NoV GI and GII in selected commodities.

Keywords: norovirus, hepatitis A virus, multiplex qRT-PCR, food contamination.

O-8

Occurrence of human enteric viruses in Spanish produce: a seventeen months study

David Rodríguez Lázaro*[1,2], Marta Díez Valcárce[3], Marta Hernández[1]

(1) Food Safety. ITACyL, Valladolid (2) Microbiology Section. University of Burgos. (3) Departamento de Biotecnología y Tecnología de los Alimentos. University of Burgos.

In this study, the prevalence of different enteric viruses in produce was evaluated. The relevance of viruses in vegetables and fruits is becoming increasingly recognised as some large outbreaks have been attributed to the consumption of raw or minimally processed leafy green vegetables contaminated with enteric viral pathogens on its surface[1,2]. To reduce the risk associated to the consumption of fresh produce, measures aiming to prevent contamination from the farm must be implemented, as well as periodical analysis of produce lots. A total of 30 lots of produce samples (5 fresh lettuces and 5 RTE lettuces per lot; each sampling time separated at least 2 weeks) were tested from October 2010 to February 2012 for the presence of human noroviruses (hNoV) genogroup I and II, and hepatitis A virus (HAV) by means of real-time RT-PCR-based methods. A full set of controls (such as sample process control -SPC-, internal amplification control -IAC-, and positive and negative controls) were used during the analytical process. All the samples were negative for HAV, whereas some samples were positive: 2 raw lettuces (1.33%) (2 lots out of 30; 6.67%) and 1 RTE lettuce (0.67%) (1 lot out of 30; 3.33%) were positive for hNoV ggI, and 3 raw lettuces (2.00%) (2 lots out of 30; 6.67%) and 3 RTE lettuce (2.00%) (2 lot out of 30; 6.67%) were positive for hNoV ggII. The results indicate the existence of a route of viral contamination via the produce supply chain, which could originate a potential public health risk.

References:

[1]Kokkinos P., Kozyra I., Lazic S., Bouwknegt M., Rutjes S., Willems K., Moloney R., de Roda Husman A.M., Kaupke A., Legaki E., D'Agostino M., Cook N., Rze utka A., Petrovic T. and Vantarakis A., 2012. Harmonised investigation of the occurrence of human enteric viruses in the leafy green vegetable supply chain in three European countries. Food and Environmental Virology 4, 179-91

[2]Kozak G.K., MacDonald D., Landry L. and Farber J.M., 2013. Foodborne outbreaks in Canada linked to produce: 2001 through 2009. Journal of Food Protection 76, 173-83

Keywords: enteric viruses; produce; prevalence; norovirus; hepatitis A virus.

O-9

Monitoring of West Nile infection in wild birds in Serbia during 2012: first isolation and characterization of West Nile Virus (WNV) strains from Serbia

Tamas Petrovic[1], Ana B. Blázquez[2], Diana Lupulovic[1], Gospava Lazic[1], Estela Escribano Romero[2], Dragan Fabijan[3], Miloš Kapetanov[2], Sava Lazic[1], Juan Carlos Sáiz[2], Teresa Merino Ramos*[2]

(1) Laboratory of Virology. Scientific Veterinary Institute "Novi Sad", Serbia (2) Departamento de Biotecnología. Instituto Nacional de Investigación y tecnología Agraria y Alimentaria, Madrid (3) Bird Protection and Study Society. Bird Protection and Study Society, Serbia.

West Nile virus is a neurovirulent mosquito-transmissible zoonotic virus, which main hosts are birds, but it also infects other vertebrates, including humans, in which it may cause sporadic disease outbreaks that may result fatal. In Europe the virus has been present for decades but, recently, the number, frequency and severity of outbreaks with neurological consequences have increased dramatically, constituting a serious veterinary and public health problem. Until 2004, only lineage 1 strains were circulating in Europe, primarily in Mediterranean countries, but since then lineage 2 strains have been isolated in different countries, becoming locally endemic and showing explosive geographic spread throughout central and south-eastern Europe. WNV circulation was addressed for the first time in Serbia in 92 blood sera and 82 pooled tissues from 134 wild resident and migratory birds from 46 species within 28 families collected during 2012 in northern Serbia. WNV antibodies were detected in 7 (7.6%) sera: 4 from Mute swans (*Cygnus olor*), 2 from White-tailed eagles (*Haliaeetus albicillas*), and 1 from a Common pheasant (*Phasianus colchicus*). Five of them neutralized WNV, but none neutralized USUV. Viral RNA was detected, for the first time in Serbia, in 9 birds: 3 Northern goshawks (*Accipiter gentilis*), 2 White-tailed eagles, 1 Legged gull (*Larus michahelis*), 1 Hooded crow (*Corvus cornix*), 1 Bearded parrot-bill (*Panarus biramicus*), and 1 Common pheasant. Phylogenetic analysis of partial E region sequences showed the presence of, at least, two lineage 2 Serbian clusters closely related to those responsible of recent outbreaks in Greece, Italy, and Hungary. Full genomic sequence from a goshawk isolate corroborated this data. These results confirm the circulation of WNV in Serbia and remark the risk of infection for humans and horses, pointing to the need of WNV surveillance programs implementation.

Keywords: wild birds, serology, neutralization, typing, lineage 2, Usutu virus, Serbia.

SESIÓN PLENARIA VI

Microbiology of extreme environments
[Joint session SEV/SEM]

CHAIRS:
Esteban Domingo
Ricard Guerrero

L-12

Life in extreme acidic habitats

Ricardo Amils[1,2]

(1) Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Madrid. (2) Centro de Astrobiología (CSIC-INTA), Torrejón de Ardoz, Madrid.

Extreme acidic habitats are different from the rest of the extreme environments because they are the product of microbial activity and not adaptations to extreme geophysical conditions of the planet. They can be found associated either to volcanic activities (sulfur world) or to the oxidation of metallic sulfides (mainly pyrite). Rio Tinto (Huelva, SW Spain) is an interesting extreme acidic environment due to its size (100 km long), constant pH (2.3) and high concentration of toxic heavy metals, conditions produced by chemolithotrophic microorganisms thriving in the high sulfidic content of the Iberian Pyrite Belt (IPB). The combined use of conventional and molecular ecology methodologies had led to the identification of the most representative microorganisms of the Tinto basin. Eighty percent of the water column diversity corresponds to microorganisms belonging to three bacterial genera, *Leptospirillum*, *Acidithiobacillus* and *Acidiphilium*, all of them conspicuous members of the iron cycle, which underlines the importance of this element in the ecosystem [1]. Recently, the characterization of the sediments and the subsurface of the IPB have started, showing a higher level of diversity that the water column. This suggests the existence of microniches in which diverse specialized metabolic activities can develop, some of them incompatible with the bulk conditions of the ecosystem (e.g., methanogenesis and sulfate reducing activities). The astrobiological, environmental and biotechnological interest of the acidic environments will be discussed from our current knowledge of these peculiar ecosystems.

[1] R. Amils, E. González-Toril, A. Aguilera, N. Rodriguez, D. Fernández-Remolar, F. Gómez, A. García Moyano, M. Malki, M. Oggerin, I. Sánchez Andrea, J.L. Sanz (2011) From Río Tinto to Mars: the terrestrial and extraterrestrial ecology of acidophiles. In "Advances in Applied Microbiology", A. Laskin, G. Gadd and S. Sariaslani (eds), volume 77, Elsevier, pp 41- 70

Keywords: chemolithotrophy, metallic sulphides, Iron cycle, Iberian Pyrite Belt.

L-13

Culture-independent analysis of viral assemblages from hypersaline environments

Josefa Antón[1]

(1) Departamento de Fisiología, Genética y Microbiología. Universidad de Alicante.

Hypersaline environments close to salt saturation harbor the highest concentration of viruses reported for aquatic systems. These environments are normally dominated by extremely halophilic members of the *Archaea* domain and a few genera of *Bacteria*, most often belonging to the *Bacteroidetes* phylum. In some case, such as the crystallizer ponds from solar salterns, the prokaryotic community is dominated by the archaeon *Haloquadratum walsbyi* and the bacterium *Salinibacter ruber* although viruses infecting these dominant hosts had not been isolated so far. Indeed, most extremely halophilic viruses available in culture infect hosts that do not seem to be ecologically relevant. To circumvent the problems associated with the culture of the relevant host-viruses pairs, our group has undertaken a polyphasic culture-independent approach to characterize the viral assemblages inhabiting hypersaline environments. Numbers and morphology of viruses are analyzed by optical and transmission electron microscopy (with Sybr Gold and negative staining, respectively) while the (meta)genomic diversity of the assemblage is characterized by means of pulsed field gel electrophoresis and sequencing of viral DNA directly retrieved from the natural samples.

Up to know, samples from coastal solar salterns in Tunisia and Spain, inland salterns in Spain and Argentina and natural salt lakes, such as the Tuz Lake in Turkey, have been analyzed. Electron microscopy has unveiled a plethora of morphologies, most of which do not correspond with any isolated virus and are similar to the many odd shapes found in other *Archaea* dominated samples. Metagenomic analyses indicated that most of the viral genome content in these samples is unique and does not present homology with the sequences available in databases, although some common traits can be found among the different analyzed hypersaline metaviromes. In spite of the low proportion of annotable genes, some interesting information regarding the "life style" of hyperhalophilic viruses can be retrieved from the sequences, such as the apparently low proportion of lysogenic viruses. Finally, we developed tools for assigning the different viral genome sequences to their putative hosts based on oligonucleotide frequencies and GC content. This hypothetical assignation could be tested by analyzing the genomes of the viruses infecting *S. ruber*, that have been very recently isolated in our lab.

Keywords: hypersaline environment, halovirus, metagenome.

L-14

Viruses in the cryosphere

Alexandre M. Anesio*[1] and Christopher Bellas[1]

[1] Bristol Glaciology Centre, School of Geographical Sciences, University of Bristol, UK.

During the summer there is significant melt on the surface of glaciers, ice caps and ice shelves when microbial communities become active and play an important role in the cycling of carbon and other elements within the cryosphere. Recent studies

have shown that viruses are found in abundance in the cryosphere and are likely to be the main controls of bacterial mortality in cold environments. In this study, viral and bacterial production was measured at the surface of arctic glaciers to address the control that viruses play in these highly truncated ecosystems. Mean bacterial carbon production associated with the debris covering icy surfaces was found to be 2.94×10^6 bacterial cells g^{-1} dry wt. h^{-1}. Virus production was found to be high. Up to 8.98×10^7 virus-like particles g^{-1} dry wt. h^{-1} were produced, which is comparable to virus production in sediments around the globe. Burst sizes were assessed by transmission electron microscopy and the mean value of 2.4 was found to be amongst the lowest recorded in the literature compared to other natural habitats. The viral induced bacterial mortality was thus calculated to be more than capable of accounting for the mortality of all bacterial production. Further to this, we found a high frequency of bacteria displaying visible virus infection (average = 23% of the bacterial community). Together with a significant correlation found between virus production and bacterial abundance, the data presented here suggests that viral induced mortality is a dominant process for the release and recycling of carbon and nutrients in supraglacial ecosystems. Because of the strong relationships between viruses and their hosts in a range of polar habitats, we argue that the high level of virus infection within the bacterial community could be key to explaining why polar regions are in fact hot spots of microbial diversity and evolution. We examine the T4-type bacteriophage community inhabiting the surface of the ablation zone of two glaciers in Svalbard alongside phages isolated from a proglacial lake at the terminus of the two glaciers. We used a molecular approach to target g23 to demonstrate that on the surface of glaciers the T4-type phages are surprisingly diverse. Phylogenetic analysis revealed that many phage g23 sequences belonged to novel groups, whilst others grouped with marine and soil phage strains, suggesting an overlap between phage communities from different biomes. Interestingly, sequences detected from the nearby proglacial lake in this study shared 99% amino acid identity to those detected in an Antarctic lake, further adding to evidence of globally distributed phage strains that may be adapted to infect specifically cold adapted hosts. Currently, we are undertaking metagenomic sequencing of the virus-only fraction of glacial communities to investigate the metabolic potential stored in the glacial virome.

Keywords: biogeochemistry, virus ecology, virus activity, glaciology.

L-15

Viruses from high temperature acidic environments

Kenneth Stedman[1,2]

(1) Center for Life in Extreme Environments, Portland State University, Oregon, USA. (2) Virus Focus Group, NASA Astrobiology Institute

Viruses found in high temperature (>70C) acidic (pH <3) environments have unique genomes and morphology ranging from turreted icosahedra to bottle-shaped [1]. The most common of these viruses are the fusiform or spindle-shaped viruses, which have been found in volcanic hot springs world-wide. The best-studied of the fusiform viruses are the SSVs, Sulfolobus spindle-shaped viruses. Strangely this fusiform morphology seems to be confined to the Archaea. The vast majority of the open reading frames in the SSV viral genomes have no similarity to other genes in public databases. This provides a unique system for the study of "extreme viruses".

Comparative genomic studies have highlighted both conserved and non-conserved genes both of which appear to have profound influences on the virus phenotype. We have also developed genetic techniques to analyze both specific and non-specific mutations in SSV genomes. Viruses and hosts isolated from throughout the world have allowed host-range studies and investigation of possible local adaptation. Finally, structural studies have highlighted the uniqueness of these viruses and indicate that their structure may be made up of hexameric subunits even though their symmetry is clearly not icosahedral. Recent progress in all of these areas will be discussed.

A recent viral metagenome survey of an acidic hot lake (>50C, pH 2) in Lassen Volcanic National Park, USA, revealed not only sequences similar to the SSVs and other known thermoacidophilic viruses, but also a unique viral genome that appears to have arisen by unprecedented DNA-RNA recombination [2]. Portions of this novel virus genome type have been detected in many environments. Recently three other complete genomes have been determined. The widespread nature of these "hybrid" viruses and a number of novel possible mechanisms for their origin will be discussed.

[1] Pina M, Bize A, Forterre P, and Prangishvili D. (2011) The archeoviruses. FEMS Microbiol Rev. 35: 1035-54
[2] Diemer GS, and Stedman KM. (2012) A novel virus genome discovered in an extreme environment suggests recombination between unrelated groups of RNA and DNA viruses. Biol Direct. 7: 13

Keywords: *Archaea*, recombination, host-range, metagenome.

CONFERENCIA DE CLAUSURA

CHAIR:
Joaquín Castilla

L-16 (Conferencia de la Ganadora del Premio "Virólogo Joven" de la SEV)

The chemokine-mediated immune response to viral hemorrhagic septicemia virus (VHSV) infection and vaccination in teleost fish

Carolina Tafalla[1]

(1) Centro de Investigación en Sanidad Animal (CISA-INIA). Valdeolmos, Madrid.

Viral hemorrhagic septicemia virus (VHSV) belongs to the *Novirhabdovirus* genus within the *Rhabdoviridae* family, and is the etiological agent of a lethal disease for many cultivated fish species worldwide, including rainbow trout (*Oncorhynchus mykiss*). In the aquatic media, the exposure to microorganisms is greater than that of terrestrial animals, and therefore, the mucosal surfaces of fish (gills, skin and gastrointestinal tract), should tolerate these microorganisms while providing a first line of defense against pathogens, triggering effective both local and systemic immune mechanisms. For this, chemokines constitute one of the first secreted immune factors upon an encounter with a pathogen that not only orchestrate immune cell recruitment, but also condition the immune response that is mounted, regulating the immune functions of target cells. Thus, chemokines have been shown to be crucial for the elimination of many different viruses. On the other hand, some viruses take advantage of chemokine regulation for replication purposes while inappropriate persistence of chemokine expression can drive tissue damage and inflammation. In this presentation, I will review our results concerning chemokine responses and subsequent recruitment of immune cells to mucosal tissues upon VHSV infection in rainbow trout. These results suggest viral mechanisms for immune evasion at the level of early chemokine response and leukocyte mobilization. Finally, I will also present our recent results concerning the characterization of early leukocyte recruitment to the muscle after DNA vaccination against VHSV. This vaccination strategy has proved very effective conferring strong and long lasting immunity against this virus. Our results reveal that there is a chemokine-mediated B cell recruitment to the muscle and therefore B cells seem to be involved in the initial phase of the immune response to intramuscular DNA vaccination. This appears to be a major difference to what we know from mammalian models where T cells play a major role and could contribute to explain the success of DNA vaccination in fish.

Keywords: fish, viral hemorrhagic septicemia virus (VHSV), chemokines, DNA vaccination.

SESIÓN PARALELA I

Immune response and vaccines

CHAIRS:
José Alcamí
Felipe García

0-10

Protection of red-legged partridges *(Alectoris rufa)* against West Nile virus (WNV) infection after immunization with WNV recombinant envelope protein E (rE)

Estela Escribano Romero*[1], Virginia Gamino[2], Teresa Merino Ramos[1], Ana B. Blázquez[1], Miguel A. Martín Acebes[1], Nereida Jiménez de Oya[1,3], Ana Valeria Gutiérrez Guzmán[2], Ursula Höfle[2], Juan Carlos Saiz[1]

(1) Department of Biotechnology. INIA. Valdeolmos, Madrid. (2) Departamento de Patología Aviar. SaBio. IREC, Ciudad Real. (3) Division of Immunology. SRSI, Milan, Italy.

West Nile virus (WNV), a neurovirulent *Flavivirus,* is maintained in nature in an enzootic transmission cycle between avian hosts and mosquito vectors, although the virus occasionally infects other vertebrates, including humans, in which it may result fatal. In Europe the virus has been present for decades but, recently, the number, frequency and severity of outbreaks have increased dramatically. To date, no licensed vaccines against WNV infection are available for bird usage. Although the cost-effectiveness of generalized vaccination programs is uncertain, the availability of WNV vaccines would certainly benefit specific populations, including birds grown for restocking, hunting activities, or alimentary purposes, and those confined to wildlife reservations, zoos, or other recreation installations. Here, we have tested the protective capability of WNV enveloped recombinant (rE) protein in red-legged partridges (*Alectoris rufa*), a gallinaceous bird widely distributed in South and Western Europe and intensively breed for hunting purposes. Partridges were intramuscularly immunized three times at 2-weeks interval with 10 mg/bird of partially purified rE protein plus adjuvant. An additional control group was sham-immunized before challenge. Except for 5 birds that were non-challenged and housed as contact controls, all birds were subcutaneously infected with 10^7 PFU of the highly neurovirulent NY99 WNV strain at our BSL-3 facilities. Oropharyngeal and cloacal swabs and feather pulp samples were collected daily up to 14 days post-infection, d.p.i. Birds were bled one week before the first immunization and at several time points during vaccination and after infection. While none of the sham-immunized birds showed anti-WNV Ab, all rE-vaccinated birds presented specific Ab after two immunizations, and most of them had nAb before challenge. Accordingly, none of the rE vaccinated partridges died, while 33.3% of the PBS-inoculated birds succumbed to the infection, as did 25% of the contact animals. Except one, all unvaccinated birds showed viremia by 3 d.p.i., being generally higher in partridges that died of WN disease. In contrast only 3 rE vaccinated birds showed viremia with very low titers. Preliminary data indicate that WNV-RNA could be detected in feathers and swabs from PBS inoculated partridges from 3 to 7 d.p.i., but not in those from rE vaccinated birds. Thus, rE vaccination fully protected partridges against WND and reduced the risk of virus spread.

Keywords: West Nile virus, vaccine, birds, E protein.

O-11

Vaccination with recombinant adenovirus expressing F or H proteins from the peste des petits ruminants virus can elicit cellular and humoral immune responses to the virus

José Rojas[1], Héctor Moreno[1], Aída García[2], Juan Carlos Ramírez[2], Noemí Sevilla[1], Verónica Martín*[1]

(1) Centro de Investigación en Sanidad Animal (CISA), INIA. Valdeolmos, Madrid (2) Unidad de Vectores Virales. Centro Nacional de Investigaciones Cardiovasculares (CNIC), ISCIII,, Madrid.

Objectives: Peste des petit ruminants (PPR) is an acute, highly contagious viral disease in small ruminants and one of the main constraints in improving small ruminant productivity. The PPRV virion contains two immunogenic integral membrane glycoproteins, the hemagglutinin (H) and the fusion protein (F), to which most of the neutralizing antibodies are directed. Adenoviruses have several attractive features to be good vectors for anti PPRV vaccine: human adenoviruses have been used for several decades as live oral vaccines in US, they can be produced inexpensively with high production, and they induce a strong immune response against vaccine antigens. Thus, we aim to construct and characterize two recombinant adenovirus, expressing the PPRV H and F proteins, and test their immunogenicity in mice.

Methods: Second-generation replication-defective human adenovirus type 5 (Ad5) were generated expressing under the EF1a promoter the H or F genes of PPRV and the red fluorescent protein (RFP) under CMV promoter. Amplified stocks in HEK293A cells, were purified and titrated using standard protocols. Groups of C57BL/6 mice were intramuscularly inoculated with $5x10^7$ ip/ml (infectious particles) Ad5-F, Ad5-H, Ad5 and PBS, respectively and boosted 3 times. Sera were collected at different times and tested for the presence of PPRV-specific antibodies by ELISA, PPRV-specific neutralizing antibodies in a plaque reduction neutralization assay and T cell specific responses evaluated by ELISPOT and proliferation assays.

Results: The insertion and expression of H and F proteins in the E1A region of the recombinant adenovirus genomes was detected by the co-expression of the RFP in HEK293A, Vero and primary ovine cells. C57BL/6 mice immunized with Ad5-H and Ad5-F elicited a high PPRV-specific IgG response evaluated by ELISA. All vaccinated mice showed PPRV-specific neutralizing antibodies and some of them show T cell responses evaluated by different assays.

Conclusion: We demonstrate that recombinant adenoviruses express the biologically active H and F proteins. Furthermore, our results indicate that intramuscular inoculation of mice with Ad5-H and Ad5-F elicited robust anti-PPRV response. Finally, the immune response and protection conferred by the successful strategies developed above will be assayed in sheep, the PPRV natural host.

Acknowledgements: This work has been supported by grants RyC-2010-06516 and AGL-2011-25025 from Ministerio de Ciencia e Innovación.

Keywords: PPRV, vaccine, recombinant adenovirus, immune system.

O-12

Protection of IFNAR (-/-) mice against african horse sickness virus serotypes 4 and 9, by heterologous (DNA/rMVA) and homologous (rMVA/rMVA) vaccination, expressing proteins VP2 and NS1

Javier Ortego*[1], Francisco de la Poza[1], Eva Calvo Pinilla[2], Francisco Mateos[1], Gema Lorenzo[1]

(1) Centro de Investigación en Sanidad Animal (CISA) INIA, Valdeolmos, Madrid. (2) Vector-borne Viral Diseases. The Pirbright Institute. Pirbright, UK.

African horse sickness (AHS) is an arthropod borne, non-contagious viral disease affecting all species of Equidae. It is caused by an Orbivirus of the family Reoviridae and is transmitted by Culicoides midges. There are nine AHSV serotypes (AHSV-1 to AHSV-9). Recent recombinant DNA technology has allowed the development of novel strategies to develop marker and safe vaccines against AHSV. We have now engineered naked DNAs and recombinant modified vaccinia virus Ankara (rMVA) expressing VP2 and NS1 proteins from and AHSV-4.

IFNAR(-/-) mice were immunized with DNA/rMVA or rMVA/rMVA expressing VP2 and NS1 proteins from AHSV-4 in an heterologous or homologous prime boost vaccination strategy. Both strategies of immunization generated significant levels of neutralizing antibodies specific of AHSV-4 in mice. In addition, ELISPOT assay showed that both vaccination strategies stimulated specific T cell responses against AHSV-4 and AHSV-9, although the response was stronger when the animals were immunized with rMVA/rMVA. Furthermore, intracellular cytokine staining assays (ICCS) showed that only the rMVA/rMVA vaccination strategy stimulated specific CD8+ T cell responses against AHSV-4 and AHSV-9. The DNA/rMVA-VP2,-NS1 immunization elicited partial protection against an homologous AHSV-4 infection and induced poor cross-protection against the heterologous AHSV-9. In contrast, IFNAR(-/-) mice vaccinated with rMVA/rMVA-VP2-NS1 were completely protected against AHSV-4 and showed high level of cross-protection when they were challenged with AHSV-9. None of vaccinated animals presented viraemia when they were challenged against the homologous AHSV-4 and very low levels when they were challenged against the heterologous virus AHSV-9.

These data suggest that the vaccination strategy based on homologous prime-boost of rMVA/rMVA expressing proteins VP2 and NS1 induce protection against infections with several serotypes of AHSV. Furthermore, the inclusion of the protein NS1 in formulations targeting this virus generates promising multiserotype vaccines against AHSV.

Keywords: AHSV, vaccine, cross-protection.

O-13

Delivery of synthetic RNA can enhance the immunogenicity of conventional anti-FMDV vaccine in mice

Belén Borrego*[1], Miguel R. Rodríguez-Pulido[2], Nuria de la Losa[1], Francisco Mateos[1], Francisco Sobrino[2], Margarita Saiz[2]

(1) Centro de Investigación de Sanidad Animal (CISA). INIA. Valdeolmos, Madrid (2) CBMSO. CSIC-UAM . Cantoblanco, Madrid.

The use of type-I interferon (IFN-I) for vaccine improvement is a strategy currently gaining attention. Besides its broad antiviral activity, IFN-I can act as a potent adjuvant for vaccination, connecting innate and adaptive immune responses, thus improving humoral and cellular immunities. This adjuvant activity can be reached not only by delivery of exogenous IFN-I, but also by its endogenous production after administration of molecules targeting the cellular receptors sensing foreign molecular patterns.

We have recently described the ability of *in vitro*-transcribed RNAs, mimicking structural domains in the non-coding regions of the Foot-and-mouth disease virus (FMDV) genome, to stimulate the production of systemic type-I IFN and induce protection against lethal doses of infectious viruses in different animal models (Rodríguez-Pulido et al., 2011, 2012). In this work, we aimed to study whether the capability of one of these synthetic non-infectious RNAs to induce IFN-I could be exploited for its use as immune adjuvant for conventional vaccination with inactivated virus.

Groups of Swiss mice were inoculated with two different FMD vaccine formulations, both based on inactivated virus, emulsified or not in a commercial adjuvant. Vaccines were given alone or in combination with the synthetic RNA corresponding to the viral IRES, which had shown to provide the best results in IFN-I induction upon intraperitoneal inoculation in mice (Rodríguez-Pulido et al., 2012). The effect of administration of the RNA at different times was also studied. The development of antibodies after a single vaccination was analysed at different times post immunization. Our results show that co-inoculation of the IRES RNA and the FMD vaccines may improve the immune response induced: specific anti-virus antibodies could be detected earlier, reached higher titers and remained for longer times after vaccination. The correlation between this immune response and protection against viral infection will be discussed.

Rodríguez-Pulido et al., 2011 "Inoculation of newborn mice with non-coding regions of foot-and-mouth disease virus RNA can induce a rapid, solid and wide-range protection against viral infection". Antiviral Res. 2011 Dec;92(3):500-4
Rodríguez-Pulido et al., 2012 "Protection against West Nile virus infection in mice after inoculation with type I interferon-inducing RNA transcripts". PLoS One. 2012;7(11):e49494. doi:10.1371/journal.pone.0049494

Keywords: vaccination, type I-IFN, adjuvant, antiviral, FMDV.

O-14

Phenotypical changes of M1 macrophages induced by dendrimer

Ana Judith Perisé Barrios*[1], Javier Sánchez Nieves[2], Javier de la Mata[2], Rafael Gómez[2], Ángel Luis Corbí[3], Ángeles Domínguez Soto[3], María Ángeles Muñoz Fernández[1]

(1) Departamento de Inmunobiología Molecular. Hospital General Universitario Gregorio Marañón, Madrid. (2) Departamento de Química Inorgánica. Universidad de Alcalá de Henares, Madrid. (3) Departamento de Microbiología Molecular y Biología de las infecciones. Centro de Investigaciones Biológicas, Madrid.

Macrophages play an indispensable role in defense, tissue repair and homeostasis. They are widely distributed and can be polarized in two main groups: M1 (classically activated macrophages) with microbicidal properties and M2 (alternatively activated macrophages) with anti-inflammatory phenotype. M1 polarization takes place at the initial stages of an inflammatory; in resolution of inflammation M2 macrophages reduced pro-inflammatory cytokine secretion. Nanomedicine is a very promising line of investigation and dendrimers could be tool to modify phenotypic and functional characteristic of macrophages.

We have screened for dendrimers and selected those that were able to modify the phenotypic and functional characteristic of M1 macrophages. Therefore, we have tested the pattern of cytokines and chemokines receptors in dendrimer treated M1 macrophages. We have studied the role and selected one dendrimer that modulate the expression of CCR2, MCP-1, MIP-1a, MIP-1b, MCP-4 and STCP-1 in M1 macrophages. Elevated levels of MCP-1 chemokine may affect HIV infection via signaling through the CCR2 receptor, since this virus would use the CCR2 receptor to infect macrophages. Both MIP-1a and MIP-1b are chemoattractants and coactivators of macrophages, those are however strictly associated with type 1 immune responses. MIP-1a and MIP-1b share also their affinity for their receptor CCR5. These results open the door to a large list of applications that can benefit from this dendrimer, especially about issues related to infections and inflammatory processes, particularly infections caused by HIV-1.

Keywords: macrophages, HIV, dendrimers.

O-15

Significant immunogenic and antigenic differences between the fusion (F) proteins of human respiratory syncytial virus (hRSV) and human metapneumovirus (hMPV): implications for vaccine development

Laura Rodríguez García*[1], Vicente Mas Lloret[1], Lorena Soledad Ver[1], Mónica Vázquez Alcaraz[1], Concepción Palomo Sanz[1], José Antonio Melero Fondevila[1]

(1) Centro Nacional de Microbiología (CNM). Instituto de Salud Carlos III. Majadahonda, Madrid.

Human respiratory syncytial virus (hRSV) and human metapneumovirus (hMPV) are important viral agents of pediatric respiratory infections worldwide. Both viruses belong to the *Pneumovirinae* subfamily of the *Paramyxoviridae* family. Protection against either hRSV or hMPV is mediated mainly by neutralizing antibodies directed against their respective fusion (F) proteins. There is only a specific treatment for hRSV available today, Palivizumab a humanized monoclonal antibody directed against the hRSV_F glycoprotein. This antibody is administered prophylactically to children at very high risk of severe hRSV infections. Palivizumab recognizes an epitope in the virus fusion (F) glycoprotein that is shared by the two conformations in which hRSV_F can fold, the metastable pre-fusion form and the stable post-fusion conformation. We have recently described a new class of antibodies specific for the pre-fusion form of hRSV_F which account for most of the neutralizing activity found in either rabbit anti-sera, raised against a vaccinia virus recombinant expressing hRSV_F or a human immunoglobulin preparation (Respigam), which was used for prophylaxis before Palivizumab[1] . Given the relevance of these findings, we have also searched for the same type of conformation specific antibodies directed against the pre-fusion form of hMPV-F. In contrast to the situation found for hRSV, the pre-fusion specific neutralizing antibodies were not found in the sera of rabbits immunized with a vaccinia virus recombinant expressing hMPV_F nor in the Respigam preparation, which also bears neutralizing antibodies against hMPV. In other words, the neutralizing antibodies raised against hMPV recognize epitopes shared by both conformations of the F protein; i.e., the pre- and post-fusion forms. These results suggest that although hRSV and hMPV are closely related viruses, the antibody immune responses to their rescpective F_proteins are very different. The implications of these differences for vaccine development will be discussed.

1.- M. Magro, V. Mas, K. Chappell, M. Vázquez, O. Cano, D. Luque, M.C. Terrón, J.A. Melero and C. Palomo (2012). Neutralizing antibodies against the preactive form of respiratory syncytial virus fusion protein offer new possibilities for clinical intervention. Proc. Natl. Acad. Sci. USA., 109, 3089-3094

Keywords: human respiratory syncytial virus (hRSV), human metapneumovirus (hMPV), antibody.

O-16

Eradication of liver-implanted tumors by Semliki Forest virus expressing IL-12 requires efficient long-term immune responses

Cristian Smerdou*[(1)], Juan R. Rodríguez Madoz[(1)], Jaione Bezunartea[(1)], Marta Ruíz Guillén[(1)], Erkuden Casales[(1)], José Medina Echeverz[(1)], Jesús Prieto[(1)], Pedro Berraondo[(1)], Sandra Hervas-Stubbs[(1)], José I. Quetglas[(1)]

(1) Departamento de Terapia Génica y Hepatología. CIMA. Pamplona.

Semliki Forest virus vectors expressing interleukin-12 (SFV-IL-12) induce potent antitumor responses against subcutaneous MC38 colon adenocarcinomas in mice. However, when MC38 tumors were implanted in liver, where colon tumors usually metastasize, SFV-IL-12 efficacy was reduced. We reasoned that characterization of immune responses against liver tumors in responder and non-responder animals could provide useful information to design more potent antitumor strategies. Remarkably, SFV-IL-12 induced high levels of circulating tumor-specific CD8 T-cells in all treated animals, which were essential for antitumor activity. However, in comparison to non-responders, tumor-specific cells from responder mice acquired an effector-like phenotype significantly earlier, were recruited more efficiently to the liver, and persisted for a longer time. All treated mice had high levels of functional specific CD8 cells at 8 days posttreatment depicted by both *in vivo* killing and IFN-gamma production assays, but responder animals showed a more avid and persistent IFN-gamma response. Interestingly, differences in immune responses between responders and non-responders correlated with the immune status of animals before treatment and were not due to the treatment itself. Mice that rejected tumors were protected against tumor rechallenge, indicating that sustained memory responses are required for an efficacious therapy. Interestingly, IL-15 receptor alpha-subunit was upregulated only in tumor-specific CD8 cells from responder animals. Finally, the combination of SFV-IL-12 with IL-15, a cytokine that sustains CD8 T cells responses, enhanced the vector antitumor effect, confirming that the antitumor activity of SFV-IL-12 against liver tumors could be potentiated by nourishing memory T-cell responses.

Keywords: semliki forest virus, cancer, gene therapy, IL-12.

O-17

Protective antiviral CD8+ T-lymphocyte memory requires N-ras

Salvador Iborra[1,2], Manuel Ramos[2], David Molina[1], Silvia Lázaro[1,2], Francisco Aguilar[2], Eugenio Santos[3], Daniel López[4], Edgar Fernández-Malavé[5]#, Margarita del Val*[1,2]#

(1) Centro de Biología Molecular Severo Ochoa (CSIC-UAM).Madrid (2) Unidad de Inmunología Viral. Centro Nacional de Microbiología, Instituto de Salud Carlos III. Madrid (3) Centro de Investigación del Cáncer, IBMCC, CSIC-USAL, Universidad de Salamanca. (4) Unidad de Procesamiento Antigénico. Centro Nacional de Microbiología, Instituto de Salud Carlos III. Madrid (5) Inmunología. Facultad de Medicina Universidad Complutense de Madrid.
#EF-M and MDV are joint senior authors.

Signals from the TCR that specifically contribute to effector versus memory CD8$^+$ T-cell differentiation are poorly understood. Using mice and adoptively-transferred T lymphocytes lacking the small GTPase N-ras, we found that N-ras-deficient CD8$^+$ T cells differentiate efficiently into antiviral

primary effectors, but have a severe defect in generating protective memory cells. This defect was rescued, although only partly, by rapamycin-mediated inhibition of mTOR *in vivo*. The memory defect correlated with a marked impairment *in vitro* and *in vivo* of the antigen-mediated early induction of T-box transcription factor Eomesodermin (Eomes), while T-bet was unaffected. Besides N-ras, early Eomes induction *in vitro* required PI3K/AKT but not ERK activation, and it was largely insensitive to rapamycin. Consistent with N-ras coupling Eomes to T-cell memory, retrovirally enforced expression of Eomes in N-ras-deficient $CD8^+$ T cells effectively rescued their memory differentiation. Thus, our study identifies a critical role for N-ras as a TCR-proximal regulator of Eomes for early determination of the $CD8^+$ T-cell memory fate.

Keywords: T lymphocytes, antiviral, memory, protection.

SESIÓN PARALELA II

Functional RNA sequences and structures

CHAIRS:
Juan Antonio García
Encarnación Martínez-Salas

O-18

Impact of divalent cations and RNA binding proteins on the RNA conformation of a picornavirus IRES element

Gloria Lozano*[1], Encarna Martínez Salas[1]

(1) Departamento de Dinámica y Función del Genoma. Centro de Biología Molecular Severo Ochoa. Madrid.

RNA structure plays a key role in internal initiation of translation. Picornavirus internal ribosome entry site (IRES) elements are cis-acting RNA regions that recruit the ribosome to internal mRNA sites. Little is known about long-range constrains determining the IRES structure. RNA folding into stable tertiary structure is dependent on the presence of divalent cations that reduce the backbone charge repulsion and stabilize the folded conformation. Likewise, RNA structure is also dependent on the binding of proteins that stabilize its functional structure. Previous studies have shown that the foot-and-mouth disease virus (FMDV) IRES element is organized in modular domains (termed 1-2, 3, 4, and 5), each of them interacting with various initiation factors (eIFs) and other RNA-binding proteins which are required for internal initiation of translation. Here, we sought to investigate the influence of these factors on the folding of the FMDV IRES using Selective 2´hydroxyl acylation by primer extension analysis (SHAPE). Our work revealed that the IRES conformation is extensively reorganized in the presence of divalent cations and RNA-binding proteins, indicating that these factors contribute to facilitate the RNA folding and to stabilize the IRES conformation. Several motifs located all along the RNA were differentially affected by the concentration of Mg^{2+} ions present in the folding buffer. Interestingly, some of these motifs are candidate regions to be involved in potential tertiary interactions. Besides, the interaction of the IRES with proteins eIF4G, eIF4B and G3BP (a novel IRES-transacting factor) induce a reorganization of its structure involving distant domains, indicating a close proximity in the native IRES structure.

Keywords: translation control, IRES, RNA structure, RNA-protein interactions.

O-19

The internal ribosomal entry site of pelargonium flower break virus specifically recruits eIF4F via the eIF4G subunit

Miryam Pérez Cañamás*[1], Olga Fernández Miragall[1], Carmen Hernández Fort[1]

(1) Departamento de Virología Molecular y Evolutiva de Plantas. Instituto de Biología Molecular y Celular de Plantas Primo Yufera. (CSIC-UPV) Valencia.

Pelargonium flower break virus (PFBV) belongs the genus *Carmovirus* in the family *Tombusviridae*. Its genomic RNA (gRNA) contains five ORFs that encode, from 5´ to 3´, two replication proteins (p27 and its readthrough product p86), two movement proteins (p7 and p12), and a protein (p37) with a dual function as coat protein and as suppressor of RNA silencing. As found for other carmoviruses, the PFBV gRNA directs translation of the replicases whereas two subgenomic RNAs are generated during infection that serve, respectively, as mRNAs for expression of the two movement proteins and of p37. Recently, we have shown that p37 can be also produced from the gRNA due to the presence of an IRES that is contained within an 80 nt segment preceding the 3´-gene. A positive correlation between IRES activity and viral infectivity was observed supporting the *in vivo* relevance of the p37 IRES-mediated translation (Fernández-Miragall & Hernández, 2011, *PLoS One* 6:e22617). In this study, we aimed to identify the initiation factor(s) (eIFs) that are required for PFBV IRES function. Addition of the IRES in *trans* to translation assays in which the PFBV gRNA was used as template resulted in strong inhibition of protein production, suggesting sequestration of relevant factors by the PFBV IRES. In agreement with this view, the *trans* inhibition was reversed by supplementing with eIF4F as well as with its subunit eIF4G, but not with eIF4E or eIFiso4F. These results together with those from RNA binding assays indicated that the PFBV IRES specifically recruits eIF4F via direct interaction with eIF4G. Remarkably, this observation parallels that reported for the IRES of *Tobacco etch potyvirus*, the unique IRES from a plant virus for which this question has been previously investigated, despite both elements are structurally very dissimilar.

Keywords: IRES, plant viruses, pelargonium flower break virus, initiation factors, viral translation.

0-20

End-to-end cross-talk in the genomic RNA of the hepatitis C virus

Cristina Romero-López* [1], Alicia Barroso del Jesús[2], Ana García-Sacristán[3,4], Carlos Briones[3,4], Alfredo Berzal-Herranz[1]

[1] Departamento de Biología Molecular. Instituto de Parasitología y Biomedicina López-Neyra, IPBLN-CSIC, Armilla, Granada. [2] Unidad de Genómica, IPBLN-CSIC. Armilla, Granada [3]. Departamento de Evolución Molecular, Centro de Astrobiología, CAB-[CSIC-INTA], Torrejón de Ardoz, Madrid. [4] Centro de Investigación Biomédica en Red de enfermedades hepáticas y digestivas [CIBERehd].

Hepatitis C virus (HCV) genome is a plus polarity, ssRNA molecule consisting of a single open reading frame (ORF) flanked by 5' and 3' untranslatable regions (UTRs) that contain structural elements essential for the execution of the viral cycle. During early infection, uncapped viral RNAs initiate their translation thanks to the action of a highly structured internal ribosome entry site (IRES) element, mainly located at the 5'UTR. This process is regulated by distant regions at the 3' end of the viral genome, such as the CRE (*cis*-acting replication element) and the 3'UTR (1-4). Through a combination of improved RNA chemical probing methods, SHAPE structural analysis and screening of RNA accessibility using antisense oligonucleotide microarrays, we have shown the existence of a direct RNA-RNA cross-talk involving the IRES element at the 5'UTR, and the CRE and

the 3'UTR regions at the end of the viral genome. The essential IRES subdomains IIIb and IIId, together with domain IV, adopted a different conformation in the presence of the CRE and the 3'UTR compared to that taken up in their absence (5). In concordance, the architecture of the 5BSL3.2 domain (in the CRE region) and the stem-loops 3'SL3 and 3'SL2 (in the 3'UTR) was significantly altered when the IRES element was added in *cis*. These observations were subsequently confirmed in a replication-competent RNA molecule. Importantly, protein factors are not required for triggering these conformational differences. Our findings point to a complex, direct and long-distance RNA-RNA interaction network that fine-tunes the conformation of essential functional domains within the HCV genome. Such an structural fine-tuning could be involved in the regulation of the recruitment of factors such as eIF3, the viral polymerase or the 40S ribosomal subunit. Our results also unveil a novel mechanism for the regulation of translation and replication, as well as for the switch between different steps of the viral cycle.

1. Bradrick,S.S., Walters,R.W. and Gromeier,M. (2006) *Nucleic Acids Res.*, 34, 1293-303.
2. Ito, T., Tahara, S. M. and Lai, M. M. (1998) *J. Virol.*, 72, 8789-96.
3. Romero-López, C. and Berzal-Herranz, A. (2012) *Cell. Mol. Life Sci.*, 69, 103-13
4. Song, Y., Friebe, P., Tzima, E., Junemann, C., Bartenschlager, R. and Niepmann, M. (2006) *J. Virol.*, 80, 11579-88
5. Romero-López, C., Barroso-del Jesús, A., García-Sacristán, A., Briones, C., Berzal Herranz, A. (2012) *Nucleic Acids Res.*, 40, 11697-713

Keywords: hepatitis C virus, internal ribosome entry site, RNA circularization, RNA estructure/function.

0-21

Identification of an RNA translational regulator element at the 3' of the TGEV genome

Silvia Márquez Jurado*[1], Aitor Nogales[1], Luis Enjuanes[1], Fernando Almazán[1]

(1) Department of Molecular and Cell Biology. National Center of Biotecnology (CNB-CSIC). Madrid.

Coronavirus (CoV) replication and transcription are complex processes that require the specific recognition of RNA *cis*-acting signals located at the ends of the viral genome. Both processes are mediated by a viral multienzymatic replicase complex encoded by the 20 Kb replicase gene together with cellular factors, whose identity and role is largely unknown. To identify cell proteins involved in CoV replication and transcription, transmissible gastroenteritis CoV (TGEV) genome ends were used as baits for RNA affinity chromatography purification. Among the identified proteins, the polypyrimidine tract-binding protein (PTB) bound preferentially to the 5' end of the genome, whereas nine proteins preferentially bound to the 3' end. These proteins included sev-

eral hnRNPs (A1, A2B1, A0, Q, and U), translation factors glutamyl-prolyl-tRNA synthetase (EPRS), arginyl-tRNA synthetase (RRS), and poly(A)-binding protein (PABP), and the p100 transcriptional co-activator. Silencing studies using siRNAs were performed to analyze the role of these proteins on CoV RNA synthesis. A functional role was demonstrated for the 3' end-interacting proteins hnRNP Q, EPRS, RRS and PABP. The RNA motifs interacting with PABP, EPRS and RRS were identified. The PABP bound specifically to the poly(A) of the genome, while the EPRS and RRS specifically bound to a 32 nt RNA motif localized at 411 nt from the 3' end of the TGEV genome. This RNA motif presented a high homology in primary sequence and secondary structure with the translation inhibitor GAIT element that is present at the 3' end of several inflammatory mRNAs. In response to IFN-gamma, the GAIT complex (EPRS, hnRNP Q, L13a, GAPDH) interacts with the GAIT element and suppresses the translation. To analyze the role of the viral GAIT motif on translation silencing, the translation of an mRNA encoding the luciferase reporter gene with the viral GAIT motif at the 3'UTR was analyzed in the presence of the GAIT complex. As previously described for the cellular GAIT element, the 32 nt viral motif inhibited the luciferase reporter gene translation. Taking in consideration these results, it could be postulated that the viral GAIT element could act either promoting the viral genome translation silencing during the translation-replication switch or interfering with the host inflammatory response to improve the efficiency of virus infection.

Keywords: coronavirus, RNA motif, translation silencing.

0-22

Diversity of cap-independent translation elements in the 3'-UTR of melon necrotic spot virus (MNSV) and their role in host range determination

Manuel Miras*[1], Verónica Truniger[1], Miguel A. Aranda[1]

[1] Departamento de Biología del Estrés y Patología Vegetal. Centro de Edafología y Biología Aplicada del Segura (CEBAS)- CSIC. Espinardo, Murcia.

In animal viruses, internal ribosome entry sites (IRES) at the 5´-untranslated regions (UTRs) of viral RNAs are the most frequently found translation control elements. Unlike animal viruses, many plant viruses (in particular those belonging to the family *Tombusviridae*) contain cap-independent translation enhancer elements at their 3´-UTRs (3´-CITEs). For *Melon necrotic spot virus* (MNSV) (genus *Carmovirus*, family *Tombusviridae*) we have shown that cap-independent translation initiation of viral RNAs is controlled by a 3´-CITE (Truniger et al., (2008) The Plant Journal 56:716-727); moreover, the activity of this 3´-CITE requires the presence of the 5´-UTR in *cis* to circularize the viral RNA through a long distance RNA:RNA interaction based in sequence complementarity. By exploring the natural diversity of MNSV, we have identified three different 3´-CITEs and showed that at least two of them have been acquired by interspecies recombination (Nieto et al., (2010) The Plant Journal 66:492-501). Interestingly, these dif-

ferent MNSV 3´-CITEs are interchangeable and confer host specificity. Thus, the 3´-CITEs of strain MNSV-264 and of strain MNSV-N confer these viruses the ability to infect melon plants otherwise resistant to MNSV (Diaz-Pendon et al., (2004) MPMI 5:223-233). Importantly, melon resistance to MNSV is controlled in the host by the eukaryotic translation initiation factor (eIF) 4E (Nieto et al., (2006) The Plant Journal 48:452-462), a component of the cap-binding complex eIF4F. In fact, our results and those of other authors suggest that the underlying mechanisms controlling host specificity include subtle and very specific interactions between the viral 3´-CITEs and the host eIF4F.

Keywords: 3´translational enhancers, cap-independent translation, RNA virus.

O-23

Distinct spatial-temporal evolution of symptomatic and non-symptomatic variants of a chloroplastic viroid

Pedro Serra*[1], Yoshiyuki Tanaka[1], Ricardo Flores[1]

[1] Department of Plant Stress Biology. Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC), Universidad Politécnica de Valencia.

As with viruses, populations of viroids (small non-protein-coding RNAs that infect plants and frequently induce disease) are composed by sequence variants that, when individually assayed, display occasionally distinct pathogenicity. However, the stability of such variants and of their associated phenotypes is a less well-understood issue. In this work we have examined what happens when a typical symptomatic variant of the *Chrysanthemum chlorotic mottle viroid* (CChMVd) is inoculated in its natural host. This variant contains an apical UUUC tetraloop with which pathogenicity is strictly associated (De la Peña et al., *PNAS USA* 1999). Our results indicate that in the first non-inoculated leaves (above that inoculated) wherein symptoms are expresed, they are not uniform: chlorotic sectors are surrounded by others asymptomatic. Upon characterization of the viroid progenies by RT-PCR, cloning and sequencing, we have observed that while variants with the UUUC tetraloop predominate in the chlorotic sectors, in the dark green sectors predominate variants with one or two mutations in this motif. We next want to know whether the latter evolve to incorporate additional mutations and finally get the GAAA tetraloop characteristic of asymptomatic variants. Conversely, inoculation with an asymptomatic variant containing this GAAA tetraloop induces a constant asymptomatic phenotype, with only variants with this same tetraloop accumulating in the progeny. In other words, symptomatic and asymptomatic CChMVd variants have a distinct evolutionary behavior. We are currently examining the molecular basis of this phenomenon carrying out, among others, competition experiments bewteen both type of variants inoculated simultaneously. We envision that the extremely high mutation rate of CChMVd, the

highest reported for any biological entity (Gago et al., *Science* 2009), combined with the ability of different variants to colonize preferentially some leaf sectors (stochastically or because they move differentially) and then induce superinfection exclusion of others, might account for the observed results.

Keywords: viroids, non-protein-coding RNAs, evolution.

O-24

Long non-coding RNAs are involved in HCV infection

Elena Carnero[1], Victor Segura[2], Nerea Razquin[1], Puri Fortes*[1]

(1) Department of Gene Therapy and Hepatology. CIMA. Pamplona (2) Department of Bioinformatics. CIMA. Pamplona.

Long non-coding RNAs (lncRNAs) play important roles in cell proliferation and differentiation. However, few studies have analyzed their role in cell homeostasis or response to extracellular agents such as viral infections. Therefore, we decided to explore the potential role of lncRNAs in Hepatitis C virus (HCV). To study this, we infected Huh7 cells with JFH-1 strain of HCV and we treated the cells or not with Interferon (IFN), used in the treatment of HCV infections. Then, we analyzed the trascriptomes of control or infected cells by microarrays and RNA seq. Transcriptome analysis revealed alterations in the expression of lncRNAs in all the samples. In the microarray, 10% of all the altered probes corresponded to lncRNAs: 892 probes for lncRNAs were significantly altered after HCV infection and 1158 in HCV infected cells treated with IFN. Altered lncRNAs are located everywhere in the genome. Some lncRNAs are intergenic while others are neighbouring coding genes which, in some cases encode for cellular proteins related to IFN pathway and/or HCV infection. We have focused on the 37 lncRNAs annotated in public databases, upregulated after infection with the highest statistical significance (B>2) and fold change (lg FC>2). We named them CSR from HCV Stimulated RNAs. We have validated the overexpression of 29 out of the 37 CSRs analyzed in HCV infected cells. Several of these are also expressed to higher levels in the livers of HCV patients than in controls. Some of the validated CSRs are upregulated after HCV infection over 100 fold. The expression of some of them is also altered by IFN. Actually, some CSR that do not respond to IFN are induced to lower levels in HCV infected cells than in infected cells treated with IFN. To determine whether some of these CSR are involved in HCV replication, we have downregulated their expression previously to HCV infection using siRNAs. Surprisingly, the decrease of CSR19, CSR20, CSR21, CSR26 and CSR34 leads to a drastic decrease in the accumulation of HCV core protein. We have studied CSR26 further and we have determined that downregulation of CSR26 leads to a decrease in viral RNA and in viral titer. Further studies will be necessary to understand the exact role of these lncRNAs in HCV infection.

Keywords: LncRNAs, HCV, IFN, antivira.l

0-25

Characterization of a tRNA-mimic domain inside the coding region of interferon alfa 5 mRNA

Rosa Díaz Toledano*[1,2], Jordi Gómez[1,2]

(1) Department of Molecular Biology. CSIC-IPBLN. Armilla, Granada. (2) Departamento de Hepatitis Virales. CIBERehd.Armilla, Granada.

RNase P is a structure-dependent ribonuclease that specifically recognizes and cleaves tRNA precursors (pre-tRNAs). Along with other tRNA metabolism enzymes, such aminoacyl tRNA synthetases, the –CCA adding enzyme or RNase Z, it has been historically used to define the presence of tRNA-like structures (Giege et al, 1998; Mans et al, 1991). Using human RNase P activity as molecular tool, we have been able to detect the presence of two tRNA mimetic motifs inside the coding region on interferon alpha 5 mRNA (IFNA5 mRNA); designated as tRNA-mimic I and tRNA-mimic II respectively.

Cleavage into tRNA-mimic I domain of IFNA5 mRNA, at positions A_{376} or A_{378}, and its minimal substrate length IFNA5(215-427), lie within the reported *IFNA1* cytoplasmic accumulation region signal (CAR), which spans positions 239 to 421 (Lei et al, 2011). Since the two IFNA subtypes have a sequence homology of 88%, we suggest that CAR region adopts a tRNA-mimic structure.

Herein we characterized tRNA-mimic I structure using enzymatic and chemical probing of three IFNA5 RNAs of increasing size: IFNA5(329-427), IFNA5(215-427), and IFNA5(197-446). Ribonucleases T1 and A were used to map unpaired nucleotides, and ribonuclease V1 was used to identify paired bases or stacked nucleotides. Single stranded positions were determinated also by lead(II)-induced cleavages, DEPC and DMS probing. Based on these data, a secondary structure is proposed, containing a pseudoknotted three-way junction similar to the one that imposes tRNA-like mimicry in the 3' end of TMV RNA (Felden et al, 1996). Additionally RNase P cleavage site lies within a large and highly resistant T1 region of the IFNA5 RNA tested fragments. This results provides a possible structural explanation for the mimicking properties of the tRNA-mimic I domain of IFNA5 mRNA.

REFERENCES: Felden B, Florentz C, Giege R, Westhof E (1996) A central pseudoknotted three-way junction imposes tRNA-like mimicry and the orientation of three 5' upstream pseudoknots in the 3' terminus of tobacco mosaic virus RNA. *RNA* 2: 201-212
Giege R, Frugier M, Rudinger J (1998) tRNA mimics. *Curr Opin Struct Biol* 8**:** 286-293
Lei H, Dias AP, Reed R (2011) Export and stability of naturally intronless mRNAs require specific coding region sequences and the TREX mRNA export complex. *Proc Natl Acad Sci U S A* 108: 17985-17990
Mans RM, Pleij CW, Bosch L (1991) tRNA-like structures. Structure, function and evolutionary significance. *Eur J Biochem* 201: 303-324

Keywords: RNA, tRNA-mimic, IFNA.

SESIÓN PARALELA III

Epidemiology and control of viral diseases

CHAIRS:
Ángela Domínguez García
Fernando de Ory

0-26

Norovirus GII.4 infections and seroprevalence of specific antibodies in Valencia, Spain

Noelia Carmona Vicente[1], Manuel Fernández Jiménez[1], Juan Manuel Ribes Fernández[1], Carlos J. Téllez Castillo[1], Javier Buesa*[1,2]

(1) Department of Microbiology. Facultat de Medicina, Universitat de València. (2) Servicio de Microbiología. Hospital Clínico Universitario de Valencia

Background and objectives. Noroviruses (NoVs) are responsible for most of the outbreaks of acute gastroenteritis worldwide, as well as a common cause of sporadic cases. NoVs are transmitted via the fecal-oral route and result in 267 million infections and over 200,000 deaths each year, mostly in infants and the elderly (Patel et al., 2009). The prevalence of different NoVs genotypes as the cause of acute gastroenteritis in the region of Valencia during a three-year period (2008-10) was investigated. In addition, the prevalence of IgG antibodies to NoV genotype GII.4 in sera from a random sample of individuals during the same period of time was determined.

Materials and methods. Baculovirus-expressed virus-like particles (VLPs) of NoV GII.4-2006b and a recombinant P2 polypeptide of NoV GII.4-2008 produced in *Escherichia coli* were used as coating antigens in enzyme immunoassays to study NoV GII.4-specific antibodies. Serum samples from 434 individuals of different ages collected between 2009 and 2010 were assayed.

Results. NoVs were detected in 42 (77.3%) outbreaks and in 7.8% sporadic cases of acute gastroenteritis. Genogroup GII strains were predominant causing both outbreaks and sporadic cases. Different genotype GII.4 viral variants were found throughout the study period (GII.4-2006a, -2006b, -2008 and -2010), being the GII.4 2010 variant the most frequently detected (40%).

Antibodies to the P2 polypeptide and to GII.4-VLP were detected in practically all (99%) of the serum samples analysed. Titers of antibodies to NoV VLPs and to the P2 domain gradually increase with age, reaching their highest concentrations in the forth decade of life.

Conclusion. These results suggest that exposure to NoV genotype GII.4 occurs early in childhood but re-infections are common.

References. Patel et al. (2009). J Clin Virol 44:1-8.

Keywords: norovirus, genotypes, antibody, prevalence.

O-27

Analysis of norovirus and sapovirus in foodborne outb reaks in Catalonia, Spain

Aurora Sabrià*[1], Rosa M. Pintó[1], Albert Bosch[1], Rosa Bartolomé[2], Thais Cornejo[2], Núria Torner[3,4], Ana Martínez[3], Mercedes de Simón[5], Ángela Domínguez[5], Susana Guix[1]

(1) Department of Microbiology. University of Barcelona. (2) Laboratory of Microbiology. Hospital Universitari Vall d'Hebron. Barcelona (3) Department of Health. Generalitat de Catalunya. Barcelona (4) CIBER Epidemiología y Salud Pública (CIBERESP). Instituto de Salud Carlos III. (5) Laboratory of the Public Health Agency. Barcelona.

Noroviruses (NoV) are highly contagious and are the leading cause of foodborne nonbacterial outbreaks of gastroenteritis in humans worldwide. Typical food items implicated in NoV outbreaks are shellfish, fruits and vegetables that have been irrigated with fecally-contaminated water, and ready-to-eat foods prepared by infected food handlers. Sapoviruses (SaV) are contained within the same family *Caliciviridae* and, although they are regarded as a cause of sporadic cases of gastroenteritis in children, they have also been involved in outbreaks.

NoV and SaV-associated gastroenteritis were investigated in outbreaks occurred in Catalonia from January 2010 to December 2012. Samples from 170 outbreaks with a suspected viral origin were screened for NoV. SaV was studied in all NoV negative outbreaks as well as in NoV outbreaks which were potentially linked to shellfish consumption. NoV was determined as the etiological agent in 129 outbreaks (75.9%), and SaV was identified in 9.8% of NoV-negative outbreaks. While NoV caused foodborne (52.0%), person-to-person (44.7%), and waterborne (3.3%) outbreaks, all SaV outbreaks were interpersonal. Of all NoV foodborne outbreaks 17 out of 64 could potentially be linked to shellfish, and more than one NoV genotype could be identified in 52.9% of them. The presence of NoV in shellfish could be confirmed in 3 samples, with values ranging from $4.1x10^4$-$9.2x10^5$ and $3.5x10^5$-$3.9x10^6$ genome copies/g of hepatopancreas for NoV GI and GII, respectively. Interestingly, 9.2% of patients from all NoV outbreaks epidemiologically linked to shellfish (5/54) were coinfected with SaV.

Samples from food handlers were collected from 155 individuals related to foodborne NoV outbreaks. Eighty-five per cent of these food handlers reported to be asymptomatic, while 15% were symptomatic. NoV shedding was detected in all symptomatic food handlers but also in 65% asymptomatic individuals. Viral load and duration of NoV shedding was studied in selected individuals, showing that duration of shedding was similar between symptomatic and asymptomatic individuals, although the latter ones shed significantly lower virus concentrations. Stools from food handlers related to NoV outbreaks were also screened for SaV, giving 1,9% of SaV shedders (3/155).

Our results confirm the importance of shellfish and infected food handlers as vehicles of NoV transmission in our country, and suggest that they may also contribute to spread SaV infections in the population.

Keywords: norovirus, sapovirus, foodborne outbreak, gastroenteritis, food handler, shellfish.

0-28

Mumps outbreaks in Castilla-León, 2012

Ana Castellanos*[1], Juan Emilio Echevarria[1], Marta Allue[2], Raúl Ortiz de Lejarazu[3], José María Eiros[4], Julio de la Puente[5], Antonia García Castro[6], José Luis Yáñez[7], Carmen Gimeno[8], Margarita García[9], María Fe Brezmes[10], Julio Ramos[11], Trinidad Parras[12], Cristina Ruiz Sopeña[13], María Eulalia Guisasola[1], Fernando de Ory[1]

[1] Departamento de Virología. Centro Nacional de Microbiología.Majadahonda, Madrid [2] Departamento de Epidemiología. Sanidad y Bienestar Social. Valladolid [3] Departamento de Microbiología. Hospital Clínico Universitario. Valladolid [4] Departamento de Microbiología. Hospital Río Hortega. Valladolid [5] Departamento de Epidemiología. Sanidad y Bienestar Social. Palencia [6] Departamento de Microbiología.Hospital Río Carrión. Palencia [7] Departamento de Epidemiología. Sanidad y Bienestar Social. Burgos. [8] Departamento de Microbiología. Hospital Santiago Apóstol. Miranda de Ebro, Burgos [9] Departamento de Epidemiología. Sanidad y Bienestar Social. Zamora. [10] Departamento de Microbiología. Hospital Virgen de la Concha. Zamora. [11] Departamento de Epidemiología. Sanidad y Bienestar Social. León. [12] Departamento de Microbiología. Hospital de León. [13] Departamento de Vigilancia Epidemiológica.DG Salud Pública. Valladolid.

Mumps is a viral childhood, vaccine preventable disease by means of measles-mumps-rubella vaccine. With the use of the vaccine, reported mumps cases decreased from 284,887 in 1984 to 1,526 in 2004. Despite of this, in the recent years are occurring mumps outbreaks affecting both vaccinated and non-vaccinated individuals. In 2012 some mumps outbreaks began in Castilla-León, being analyzed five of them, occurred in the provinces of León (#1, 5 cases, 16 samples), Palencia (#2, 85, 160), Valladolid (#3, 113, 260), Burgos (#4, 16, 35) and Zamora (#5, 8, 17). A total of 488 samples from 227 cases (183 single and 50 paired serum samples, 92 urine samples, 160 pharyngeal exudates [PE] and 2 cerebrospinal fluids [CSF]) were studied. The presence of mumps RNA was determined by polymerase chain reaction (PCR) (Royuela et al, Journal of Clinical Virology 52; 2011:359-262) in PE, urine and CSF samples; the genotype was determined in positive samples. Qualitative (for IgM) and quantitative (for IgG) determinations of specific mumps antibodies were done by indirect ELISA (Enzygnost, Siemens, Germany).

Globally, positive result was obtained in 161 cases (70.93%), 39 with single positive result by PCR, 49 with single positive result by serology, and 73 by both diagnostic approaches. In relation to the sample studied positive results were obtained for 112 PE (70%), 11 urines (11.96%) and 105 serum samples (50.48%). Among those with single sample, IgM was detected in 40 (21.86%), and high titer of IgG 5000 in 88 (48.09%). Amongst the 25 paired serum samples, a significant rise in the IgG

titer was obtained in 6 (24%), and a titer 5,000 in 11 (44%). The genotype detected in all cases was G, as expected, since it is the dominant one in Spain from 2005.

As conclusions, the most adequate sample for direct diagnosis of mumps virus is the PE, better than urine sample. In the current epidemiological scenario the detection of specific IgM fails in an important number the cases, being useful the quantitation of specific IgG. The use of paired serum samples highly improved the serological diagnosis.

Keywords: mumps, PCR, serology.

0-29

Molecular characterization of three outbreaks of mumps in Asturias. Isolation of the strains circulating in the latest epidemic wave in human lung carcinoma A-549 cells

José A. Boga*[1], Óscar Martínez[1], Marta E. Álvarez[1], Susana Rojo[1], Ana Palacio[1], Santiago Melón[1], María de Oña[1]

(1) Servicio de Microbiología. Hospital Universitario Central de Asturias. Oviedo.

Objectives: To characterize mumps virus (MuV) causing three outbreaks occurred in Asturias in 2002 (B1), 2006/2007 (B2) and 2012/2013 (B3). To check that B3 strain effectively replicates on human cells causing cytopathic effect (CPE).

Material and methods. B1**:** 295 samples of 216 patients (23.3 ± 13.8 years, 2-87 years) between January and September 2002, B2: 144 samples of 119 patients (27.1 ± 13.6 years, 11-87 years) between December 2006 and April 2007, and B3: 924 samples from 1 January 2012 to 15 January 2013 of 625 patients (21.4 ± 13.4 years, 1-87 years) with suspected mumps were collected.

Samples were inoculated in Vero and MRC-5 cells (shell vials and conventional tubes). To detect and genotype MuV, viral RNA previously extracted, was amplified by two "in house" nested RT-PCRs directed against NP and SH genes, respectively. SH amplicons obtained from 14 samples B1, 8 B2 and 30 B3 were sequenced and compared with homologous regions of representative isolates of different genotypes. 44 strains with CPE were inoculated in A-549 cells and observed periodically during 2 weeks to visualize syncytia formation. To confirm that CPE was caused by MuV, an IFA with anti-Mumps Mabs (Chemicon) was performed.

Results: MuV was detected in 89 (30.2%) samples of 85 (39.4%) patients (21.1 ± 9.5 years, 11-87 years) of B1, in 67 (46.5%) of 61 (51.3%) patients (21.9 ± 9.2 years, 7-53 years) os B2 and in 397 samples (43%) of 352 (56.4%) patients (20.8±9.6 years, 1-63 years) of B3. The sensitivity of the techniques was 94.8% for PCR , 54% culture and 38,4% shell-vial ($p < 0.0001$). While B1 and B2 strains belonged to type H and subtype G6, respectively during B3 co-circulated strains belonged to subtypes G5 and G6. The analysis of the cultures of the 44 strains of MuV in A-549 cells

showed that CPE was detected at 5, 10 and 15 days, identifying MuV by IFA in 34.1%, 62.8% and 82% of subcultured strains, respectively.

Conclusions. 1) The last epidemic wave of MuV has been the longest. 2) The PCR is the most sensitive technique. 3) The three outbreaks were caused by different genotypes of MuV (H, G5 and G6). The fact that they are different from that used in the vaccine (genotype A) could suggest that the vaccine does not appear to confer protection against certain non-vaccine genotypes. 4) MuV can also be isolated in human cells, such as human lung carcinoma A-549 cells.

Keywords: mumps virus, genotyping, cell culture.

O-30

Molecular epidemiology of influenza and other respiratory viruses from 2006-2007 to 2011-2012 seasons in Catalonia, Spain

Andrés Antón[1], Nuria Torner[2], Ricard Isanta[1], María Ángeles Marcos[1], Marta Camps[1], Patricia de Molina[1], Ana Martínez[2], María Teresa Jiménez de Anta[1], Tomàs Pumarola[1]

[1] WHO National Influenza Centre - Facultad de Medicina. Universidad de Barcelona. [2] Department de Salut. Generalitat de Catalunya. Barcelona.

INTRODUCTION/OBJECTIVE: Respiratory tract infections by influenza and other respiratory viruses represent a substantial public health burden. The circulation of influenza and other respiratory viruses from the 2006-07 to 2011-12 seasons in Catalonia is described. MATERIAL/METHODS: From week 40/2006 to week 20/2012 nasopharyngeal samples were collected from patients with influenza-like illness through the Catalan Influenza Surveillance Network. Cell culture and molecular PCR-based methods were used for the detection, typing and subtyping of respiratory viruses. Phylogenetic analyses and molecular characterisations of HA, NA and M2 were performed from a representative sampling. RESULTS: 6323 nasopharyngeal specimens were collected, of which the 64% were laboratory-confirmed for: FLUAV (40%), HRV (22%), HAdV (14%), FLUBV (13%), HRSV (9%), HCoV (5%), HEV (5%), HPIV-2 (3%), HPIV-3 (3%), HPIV-1 (2%), HPIV-4 (2%) and FLUCV (<1%). Influenza epidemics were reported every season although a particular type and FLUAV subtype usually became the predominant. Phylogenetic analyses of influenza viruses revealed a great genetic diversity among circulating strains, including some intra-clade reassortants. The predominant FLUBV lineage was different from that included in the recommended vaccine in 3 out of 6 seasons. Molecular characterisation allowed to monitor the acquisition of resistance mutations to adamantans and to neuraminidase inhibitors. DISCUSSION/CONCLUSIONS: FLUAV was the respiratory virus mostly reported during all seasons with annual predominance of a particular subtype, followed by HRV, HAdV, FLUBV and HRSV at varying levels. The inaccurate prediction of the predominant FLUBV lineage supports the formulation of a quadrivalent

influenza vaccine including both FLUBV lineages. Molecular characterisation of HA, NA and M2 revealed the circulation of a great number of genetic variants with the progressive acquisition of mutations, also within HA antigenic epitopes and NA enzymatic active site, that were fixed along time. The progressive increase of resistance to adamantans among current circulating FLUAV subtypes and to oseltamivir among influenza A(H1N1) virus were also reported.

Keywords: influenza; surveillance, respiratory viruses.

0-31

Molecular epidemiology and evolution of human respiratory syncytial virus B, BA genotype, in spanish hospitalised children

Ana Calderón[1], Alfonsina Trento[2,3], María Luz García García[4], Cristina Calvo[4], Mónica González Esguevillas[1], Mar Molinero[1], Silvia Moreno[1], María Teresa Cuevas[1], Francisco Pozo[1], José Antonio Melero[2,3],Inmaculada Casas*[1]

(1) Influenza and Respiratory Virus Unit. National Center of Microbiology, CNM, ISCIII. Majadahonda, Madrid (2) Biología Viral Unit. National Center of Microbiology, CNM, ISCIII. Majadahonda, Madrid (3) CIBERES. CIBER de Enfermedades Respiratorias. (4) Department of Pediatrics. Hospital Severo Ochoa, Leganés, Madrid.

Introduction. HRSV is recognized as the most common cause of LRTI-related hospitalization of children <1 year of age. Groups A and B are sero- and genetically distinguishable. Genetic differences are mainly located in the G glycoprotein. Phylogenetic studies identified numerous genotypes in both A and B groups and demonstrated a complex circulation pattern during the same annual season. A new HRSV-B genotype (BA), with a 60 nt duplication after residue 791 of the G protein gene has replaced all others B genotypes. Nt changes over time have led to the emergence of 6 lineages in the BA genotype (BA-I to VI)

Objectives. To improve our knowledge of the molecular epidemiology of HRSV-B, BA genotype, by analyzing the G protein complete gene of circulating viruses in Madrid. To define clinical details from patients infected with viruses classified in different BA lineages

Methods. A total of 2075 NPAs from hospitalized children <2 years were collected from Sept 2008 to June 2011. HRSV-A and B detection was made by multiplex RT-PCR that rendered 408 positives. A subset of 48 HRSV-B positive were randomly selected and the G protein gene 60 nt duplication was detected by rt-RT-PCR. 35 full-length G protein genes were amplified and sequenced. Phylogenetic trees were generated by ML with the TN+G as best fit model of evolution using bootstrap 1000 resampling analysis. By Bayesian MCMC method, rates of nt substitution/site, time of MRCA, and the demographic history, were estimated

Results-Conclusions. The whole G gene phylogenetic analysis among BA viruses, 57 strains from Madrid, 53 viruses worldwide, and 20 reference genotype strains (BA-I to IV and VI), revealed that sequences from Madrid clustered in 4 lineages (MAD-I to IV). Sequences from early seasons clustered in MAD-I and II and since 2005 in MAD-III and IV which confirms the replacement of dominant local lineages over time. Analysis of the Madrid sequences with the worldwide BA genotype showed that MAD-I clustered with reference strains of BA-I, II, and III lineages and MAD-II, III and IV clustered into BA-IV which had a 6 nucleotide deletion at position 490 and replacing the original B group

Directional evolution of the BA lineages from 1998 to 2011 shows the clades corresponding to early genotypes (BA-I to III) have been replaced since 2005 by the predominant BA-IV

Patients infected with MAD-IV viruses presented a higher rate of hypoxia (72.9%, p=0,001), which could be associated with greater severity

Keywords: human respiratory syncytial virus, molecular epidemiology, G protein gene, children <2 years.

0-32

Viral characteristics in prolonged shedding of influenza A(H1N1)pdm09 virus and clinical outcome in patients admitted to intensive care units

Francisco Pozo*[1], Noelia Reyes[1], Alejandro González Praetorius[2], María Huertas[3], Alicia Beteta[4], Juan Ledesma[1], Mayte Cuevas[1], Ana Calderón[1], Mónica González Esguevillas[1], Mar Molinero[1], Inmaculada Casas[1]

(1) Influenza and Respiratory Virus Unit. Instituto de Salud Carlos III. Majadahonda, Madrid (2) Microbiology Section. University Hospital of Guadalajara. (3) Microbiology Service. Hospital General La Mancha. Alcázar de San Juan. Ciudad Real(4) Microbiology Unit. Hospital Nuestra Señora del Prado. Talavera de la Reina. Toledo.

Objective. Several clinical factors including the presence of major comorbidities, a delay in starting antiviral treatment and ongoing respiratory symptoms after 5days of antiviral treatment, have been reported to promote prolonged viral shedding of the A(H1N1)pdm09 virus and a worse outcome in patients admitted to intensive care units (ICUs). However, virological factors have been less studied. In order to ensure an effective management of newer strains of influenza viral infections it is essential to accumulate evidence regarding both clinical and virological patient's intensive care parameters.

Methods. Respiratory specimens from 15 confirmed A(H1N1)pdm09 patients admitted to ICUs of 3 hospitals in CastillaLaMancha (Spain) were subsequently analyzed for viral load quantitation, as well as for the presence of hemagglutinin mutations and antiviral susceptibility until a negative result was obtained. Measurement of viral load in respiratory specimens was carried out using an in-house developed quantitative real time RT-PCR including an endogenous internal control for normalizing the values. Hemagglutinin and neuraminidase genes were amplified by conventional nested RT-PCR methods and sequenced by Sanger dideoxy technology for checking for the presence of D222G/N/E and the H275Y and N295S mutations respectively

Results and Conclusions. A(H1N1)pdm09 was detected in all first pharyngeal aspirates taken from all 15 patients with viral load ranging from less than 10 to 138461 (reported as the number of influenza virus genomes per 10 000 ribonuclease P copies). A total of 47% of the patients remained positive in a second respiratory specimen (+7 days) taken after oseltamivir treatment was started. Viral load was lower compared with first specimens except for one patient with a viral load 60 times higher. Only one patient persisted positive for A(H1N1)pdm09 fourteen days after admission in ICU despite oseltamivir treatment. No H275Y mutation was observed in any of the viral strains studied. Six patients (40%) were infected with viral strains bearing a mutation at position 222 in the hemagglutinin gene (H1 numbering): D222G (3), D222E (2) and D222N (1). Although D222G/N mutations have been associated with severity of A(H1N1)pdm09 infections or adverse outcomes by enhancing the binding capacity of 2-3 sialyl receptors to viruses, they seem not to be involved in prolonged shedding of the virus, nor is the initial viral load detected in patients admitted to ICUs.

Keywords: influenza, ICU patients.

0-33

Epidemiological surveillance of resistance to antiretroviral drugs in patients newly diagnosed of HIV-1 infection in 2004-2012: role of transmission clusters in the propagation of resistant strains

Yolanda Vega*[1], Elena Delgado[1], Miguel Thomson[1], Aurora Fernández García[1], Teresa Cuevas[1], Vanessa Montero[1], Francisco Díez Fuertes[1], Ana María Sánchez[1], Lucía Pérez Álvarez[1], Study Group New HIV Diagnoses, Galicia, Basque Country[1]

(1) Department of HIV Biology and Variabiliaty. Instituto de Salud Carlos III. Majadahonda, Madrid.

Objective: To study the role of transmission clusters(TC) in the propagation of resistance to antiretroviral drugs in patients newly diagnosed of HIV-1 infection in Galicia and Basque Country in 2004-2012.

Patients and Methods: A total of 1628 patients diagnosed of HIV-1 infection in 2004-2012 in Galicia (n=682) and Basque Country (n=946), Spain, were included in the study. Resistance mutations to reverse transcriptase inhibitors (RTI) and protease inhibitors (PI), defined for purposes of surveillance of drug-resistant strains (SDRM),

using Calibrated Population Resistance (CPR) application of HIVdb Program, Stanford University (http://sierra2.stanford.edu/sierra/servlet/JSierra), were identified. The phylogenetic analysis for the study of TC was done with the maximum likelihood method implemented in RAxML.

Results: A total of 131 (8%) sequences had at least one SDRM to RTI and 46 (2.6%) to PI, with the overall frequency being similar in both regions. Sixty nine TC in Galicia and 76 in Basque Country were identified, with a remarkably large cluster of subtype F1 in Galicia comprising 62 individuals. Nearly 40% of 131 sequences with resistance mutations were included in 27 TC. Among the viruses of Galicia, prominent TC were four of subtype B, currently expanding, which had mutations associated to high level resistance to nonnucleoside RTI (NNRTI) (K103N/S, Y188L, G190A), four with resistance to nucleoside RTI (NRTI) (M41L, L210W, T215CS, M184I), and one, also expanding, with resistance mutations to both NRTI and NNRTI (T215CN, K103N, Y188L). These 9 clusters comprise mostly homosexual men from Galicia. On the other hand, in Basque Country there was a notable increase in frequency of resistance to PI from 2010 (2%) to 2012 (10%), associated with transmission of L90M mutation in two TC, of subtypes B and C, respectively, mostly among homosexual men in that region.

Conclusions: The study of transmission clusters as a tool for epidemiological surveillance of drug resistance in new HIV-1 diagnoses allows to know the propagation of resistant strains according to population groups and to identify those exhibiting more rapid expansion. This information is of particular relevance, not only for the prescription of the most appropriate antiretroviral therapeutic regimen to each patient, but also for informative campaigns on preventive measures on the population at risk of acquiring HIV-1.

Keywords: HIV, newly diagnosed, transmission cluster, transmitted resistance.

0-34

Origin of Dengue virus type 1 from autochthonous outbreaks in Europe 2012-2013

Leticia Franco*[1], Lieselotte Cnops[2], Ivan Kurolt[3], Marjan Van Esbroack[2], Francisca Molero[1], Lourdes Hernández[1], Antonio Tenorio[1]

(1) Department of Arbovirus and imported viral diseases. CNM.ISCIII. Majadahonda. Madrid (2) Department Clinical Sciences Central Laboratory for Clinical Biology. Institute of Tropical Medicine. Antwerp, Belgium (3) Research Department . University Hospital for Infectious Diseases "Dr Fran Mihaljevic". Zagreb, Croacia.

Dengue remains as the major arboviral threat for humans. The disease is caused by 4 different but antigenically related viruses, DENV-1 to DENV-4, transmitted to humans through the bites of *Ae. aegypti* and *Ae. albopictus* mosquitoes. The disease is endemic in Asia, America, Africa and Oceania.

In Europe, the last recorded deadly epidemic was in Greece and in other Mediterranean countries including Spain in 1928. In that period, *Ae. aegypti* was the implicated vector.After the World War II, this efficient dengue vector disappeared from Europe, but another competent vector, *Ae. albopictus,* introduced in Albania in late 70s, is now distributed in over 10 European countries, including the Mediterranean coast or Spain. More recently,*Ae. aegypti* was reintroduced in south Russia and in 2005 is introduced in Madeira.

In 2010, after more than 80 years without activity in Europe, dengue re-emerged in the French Riviera and Croatia, with small outbreaks. Two years later, in October 2012, a sustained and explosive epidemic appeared in the Madeira Island, with 2242 reported infections, including 78 visitors from 13 European countries. In this study we attempted to identify the most probable source of origin of the virus introduced in Madeira taking advantage of the ongoing collaborative European initiative on imported dengue and chikungunya infections in Europe. Since December 2012 we received samples from 4 acute cases with confirmed diagnosis of dengue fever after visiting the Madeira Island .Complete envelope sequencing and posterior phylogenic analyses revealed that the Madeira strains belong to DENV- 1, genotype V, Latin-American cluster, Lineage 1 (L1) , grouping with strains recently isolated in Brazil (2008, 2010 and 2011) and Colombia (2005 to 2008). Aside from the autochthonous cases of dengue fever in southern France and Croatia, the Madeiran epidemic also has occurred by DENV-1 circulation. Phylogenetic analysis revealed that all of them belong to different lineages of the American /African genotype V. France strain appears to be similar to those circulate in Martinique and Croatian DENV-1 clustered with Indian strains.

Finally due to intense traffic of tourists and Brazilian natives residents in Madeira , and according to our results, we could hypothesized that the most probable origin for the Madeiran DENV 1 strain ,could be a Brazilian virus that circulated in the north part of the country near to the Colombia and Venezuela border in recent years.

Keywords: dengue , Madeira, outbreak.

0-35

Epidemic adenoviral cojunctivitis. Detection of the source of infection of an outbreak in an ophtalmological service

María de Oña*[1], Marta E. Álvarez Argüelles[1], José A. Boga[1], Óscar Martínez[1], María Torralba[1], Santiago Melón[1]

(1) Servicio de Microbiología. Hospital Universitario Central de Asturias. Oviedo.

Introduction. Most outbreaks of epidemic conjunctivitis caused by adenovirus (ADV) have a common source of infection (i.e., an ophthalmological clinic or a pool). Secondary cases can also occur in family, social and work environments.

Objectives. To study the conjunctivitis diagnosed

in 2012 and determine the source of adenoviral infection in an ophthalmological service.

Patients and samples. During 2012, 90 conjunctival swab samples, 1 intraocular fluid and 1 corneal scraping belonging to 85 patients [17 children (mean age 5.9±4.1 range 1-13) and 68 adults (mean age 50.8±17.9 range 19 -93 years)] were processed. The patients came from ophthalmological service (28), emergency medical service (12), other services (10) and other hospitals (26). The clinical diagnosis was conjunctivitis (76 cases), keratoconjunctivitis (5) and endophthalmitis (4). The higher number of samples were received on February and June (16 and 17, respectively). 54 samples taken from devices and eyewashes of the ophthalmological service were also received. Human samples were inoculated in Vero and MRC-5 cells (shell vials and conventional tubes) according to the lab protocol. Viral DNA was extracted from all samples. Two "in house" real time-PCRs were used to detect ADV (RT-PCR1), and HSV1, HSV2 and VZV (RT-PCR2). ADVs were genotyped by sequencing the amplicons and comparation with homologous regions of representative isolates of different genotypes.

Results. ADV was detected in 39 (45.8%) patients [35 adults and 4 children]. The higher number of cases came from the ophthalmological service (64.2% of the consultations and 46.1% of positives) and were concentrated mostly on February. HSV1 was detected in 9 patients [3 children and 6 adults] and HSV 2 in 2 patients with endophthalmitis and conjunctivitis with pseudomembrane formation. ADV was detected in 20 of the 35 adult patients by both techniques, 10 were positive only by cell culture and 5 only by PCR. ADV was also detected in 3 objectives and 3 controls of the devices from the ophthalmological service. The molecular characterization of 19 isolates showed that all cases were genotype Adenovirus 8.

Conclusions. 1. A source of adenoviral infection in ophthalmoscopes used in ophthalmology was identified.2. Viral detection by RT-PCR was the best diagnostic method by the speed of diagnosis, as well as the determination of the source of the outbreak.3. Adenovirus type 8 was responsible for conjunctivitis diagnosed in Asturias in 2012.

Keywords: adenovirus, genotyping, outbreak.

SESIÓN PARALELA IV

Virus evolution

CHAIRS:
Cecilio López-Galíndez
Jesús Navas

O-36

Evolutionary dynamics of genome segmentation in multipartite viruses

Jaime Iranzo*[1], Susanna Manrubia[1]

(1) Departamento de Evolución Molecular. Centro de Astrobiología (INTA-CSIC) Torrejón de Ardoz, Madrid.

Multipartite viruses are formed by a variable number of genomic fragments packed in indepedent viral capsids. This fact poses stringent conditions on their transmission mode, demanding, in particular, a high multiplicity of infection (MOI) for successful propagation. The actual advantages of the multipartite viral strategy are as yet unclear, and, consequently, the origin of multipartite viruses represents an evolutionary puzzle. While classical theories suggested that a faster replication rate or higher replication fidelity would favour shorter segments, recent experimental results seem to point to an increased stability of virions with incomplete genomes as a factor able to compensate for the disadvantage of mandatory complementation [1]. Using as main parameters differential stability as a function of genome length and MOI, we calculate the conditions under which a set of complementary segments of a viral genome could outcompete the non-segmented variant. Further, we examine the likeliness that multipartite viral forms could be the evolutionary outcome of the competition among the defective genomes of different lengths that spontaneously arise under replication of a complete, wild-type genome [2]. We conclude that only multipartite viruses with a small number of segments could be produced in our scenario, and discuss alternative hypotheses for the origin of multipartite viruses with more than four segments [3].

[1] Ojosnegros S. et al. (2011) Viral genome segmentation can result from a trade-off between genetic content and particle stability. *PLoS Genet.* 7: e1001344

[2] García-Arriaza J, Manrubia SC, Toja M, Domingo E, Escarmís C. (2004) Evolutionary transition toward defective RNAs that are infectious by complementation. *J. Virol.* 78: 11678-11685

[3] Iranzo J, Manrubia SC. (2012) Evolutionary dynamics of genome segmentation in multipartite viruses. *Proc. R. Soc. Lond. B* 279: 3812-3819

Keywords: segmented viral genomes, multiplicity of infection, complementation, mathematical model.

O-37

Foot-and-mouth disease virus response to the mutagenic analogue 5-fluorouracil

Ignacio de la Higuera*[1], Kamlendra Singh[2], Macarena Sierra[1], Stefan G. Sarafianos[2], Esteban Domingo[1]

(1) Department of Virology and Microbiology. Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM).Cantoblanco, Madrid. (2) Christopher S. Bond Life Sciences Center, Department of Molecular Microbiology & Immunology. School of Medicine, University of Missouri. Missouri. Columbia, USA.

5-Fluorouracil (FU) is a pyrimidine base analogue whose nucleotide derivative 5-fluorouracil-triphosphate (FUTP) displays a dual inhibitory and mutagenic activity on the picornavirus foot-and-mouth disease virus (FMDV) (Agudo et al. J. Mol. Biol. 2008 Oct 10; 382(3): 652-66): 1) It inhibits the uridylylation of the RNA synthesis primer protein VPg, and 2) it is incorporated in the viral RNA during elongation, causing an increase in the mutation frequency of the viral population. Although picornaviruses resistant to the purine analogue ribavirin (Rib) have been described, no mutants resistant to FU have been reported. Here we describe the isolation and characterization of a FMDV mutant displaying decreased sensitivity to FU. Passage of FMDV in the presence of Rib resulted in selection of a population that included the Rib-resistant substitution M296I in its polymerase (3D) (Sierra et al. J. Virol. 2007 Feb; 81(4): 2012-24). When this FMDV population was then passaged in the presence of FU, M296I reverted to wild-type while a population with V173I in its polymerase was selected. An infectious clone expressing V173I in 3D displayed decreased fitness in the absence of FU but increased fitness in the presence of FU. The reconstructed mutant V173I virus produced mutant spectra with lower complexity than the wild-type virus, upon replication in the absence or presence of FU. Biochemical studies with purified 3D indicate that the mutant displayed a 6-fold higher preference for incorporation of U rather than FU opposite to A in the template than the wild-type enzyme. Also, when FU was present in the template RNA the selectivity of the V173I mutant 3D in favour of incorporating A instead of G was 8-times higher than for the wild-type 3D. Further studies on the template-copying fidelity of these enzymes are in progress. In conclusion, we have isolated the first pyrimidine analogue-resistant FMDV mutant, with substitution V173I in 3D, and the virological and enzymatic results suggest that this substitution increases viral fitness in the presence of FU, and attenuates the mutagenic activity of FU. The availability of FMDV mutants displaying resistance to pyrimidine and purine mutagenic analogues offers a new tool to investigate antiviral designs based on lethal mutagenesis.

Keywords: foot-and-mouth disease virus, 5-fluorouracil, RdRP, lethal mutagenesis.

O-38

Effect of mutational increase on the recognition of RNA structural motifs in 5' genomic region of the hepatitis C virus by biochemical factors

Samuel Prieto Vega*[(1)], Celia Perales[(2,3)], Esteban Domingo[(2,3)], Sunnie Thompson[(4)], Jordi Gómez[(1,3)]

(1) Departamento de Biología Molecular. Instituto de Parasitología y Biomedicina López Neyra. CSIC. Armi-

lla, Granada. (2) Centro de Biología Molecular Severo Ochoa (CSIC-UAM). Cantoblanco, Madrid. (3) Centro de Investigación Biomédica en Red de enfermedades hepáticas y digestivas (CIBERehd). (4) Department of Microbiology. University of Alabama. Birmingham, USA.

The 5' region of the genome of the hepatitis C virus (HCV) RNA is very rich in structural elements that can be identified *in vitro* by biochemical and biophysical factors, such as for example, by interaction with the hepatic micro RNA miR-122 and the ribosomal 40S subunit, or by sensitivity to RNases III and P, and to UV light (254nm). These motifs involve regions of primary, secondary and tertiary structure of the RNA.

The primary objective was to assess the effect of the mutational increase in populations of RNA molecules in the region 1-570 of the HCV genome, in the identification of structural motifs by the factors outlined above. A protocol of mutagenic PCR and *in vitro* transcription was used to generate mutated RNA populations. The second objective was to evaluate the number of mutations that accumulate in this region of the viral RNA before reaching error catastrophe threshold in a viral culture in the presence of ribavirin.

The effect of the incorporation of mutations in populations differs depending on the structural levels and on the individual structural motifs. They are especially detrimental in recognition sequences of miR 122 (primary structure), the tRNA-like structure (tertiary structure) for RNase P, and the secondary structure that determines a conformational change in 1-570 RNA for RNase III. The mutational effect on the structure of 1-570 RNA was also analysed in native gels and with single and double chain RNases, RNases T1 and V1, respectively. The conformational dispersion gradually increases with the number of mutations; however, resistance to RNases shows a more complex behaviour for which we as yet have no explanation. Overall, the experimental analysis of the sensitivity of different structural motifs in the context of a long RNA modifies the concept that was established theoretically with isolated structural motifs, for example, the robustness of the secondary structure to mutation being clearly depending on the RNA context.

The mutational load at which 50% loss of identification of each of the structures is reached is lower than the value that is achieved with treatment of HCV with ribavirin in cell culture, but overall, the effect of the increase of mutation on all structural motifs makes plausible the hypothesis that the functions carrying these structures could be sensitive to therapeutic strategies based on error catastrophe.

Keywords: quasispecies, RNA-structure, error-threshold.

O-39

Experimental evolution of tobacco mosaic virus in *Arabidopsis thaliana* plants with altered cytoskeleton dynamics

Inmaculada Ferriol[1,2], Eduardo Peña[3], Adrián Sambade[4], Henrik Buschmann[5], Annette Niehl[6], Santiago F. Elena[7,8], Manfred Heinlein[3,6], Luis Rubio*[1,9]

(1) Departamento de Protección Vegetal y Biotecnología. Instituto Valenciano de Investigaciones Agrarias. Moncada, Valencia (2) Department of Plant Pathology. University of California Davis. USA (3) Departement Integrative virology. Institut de Biologie Moléculaire des Plantes. Université de Strasbourg. France (4) Department of Comparative Neurobiology. Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València. Patterna. Valencia (5) Institute for Botany. University of Osnabrück. Germay (6) Botany Department of Environmental Sciences. University of Basel. Switzerland (7) Instituto de Biología Molecular y Celular de plantas, CSIC-UPV. Valencia (8) Institute the Santa Fe.New Mexico. USA. (9) Departamento de Protección Vegetal y Biotecnología. Instituo Valenciano de Investigaciones Agrarias. Moncada Valencia.

The cytoskeleton is an interconnected network of filamentous polymers and regulatory proteins involved in cell division, cellular scaffolding, and in the determination of cellular behavior, signaling and fate. Microtubules (MT), one type of these tubular polymers, have been shown to be involved in Tobacco mosaic virus (TMV) RNA movement suggesting the existence of MT-guided RNA localization and movement mechanisms in plants. We wondered what would be the effect of altering the MT dynamics in TMV infection. Would virus fitness decrease or increase? Would TMV adapt to these changes of the cell? Could the study of the TMV response to these cellular modifications provide insight on the cytoskeleton functioning? To answer these questions we set up an evolutionary experiment based on serial passages of TMV through two *Arabidopsis thaliana* mutants with reduced microtubule dynamics: *tortifolia 1*, a knockout mutant of the microtubule associated protein TOR1 gene, and *tortifolia 2*, a replacement of Arg-2 with Lys in the -tubulin 4 protein. As control the same serial passages were performed in *A. thaliana* wild type plants. We found adaptive mutations in the TMV replicase gene that increased the viral fitness (measured as infectivity and/or accumulation) in the mutant *A. thaliana*plants, whereas they had not effect or decreased fitness in *A. thaliana* wild type plants and *Nicotiana tabacum* protoplasts. These findings indicate a great adaptability of TMV to subtle alterations in the MT network and the functional implication of the viral replicase domain into intracellular and intercellular viral RNA movement. It also provides a tool to investigate the otherwise elusive role of the proteins involved in the cytoskeleton dynamics regarding MT-supported RNA movement in plants.

Keywords: adaptation, fitness, interaction, microtubules, TMV, mutants.

O-40

An unbiassed genetic screen reveals the polygenic nature of the influenza virus anti-interferon response

Maite Pérez-Cidoncha[1], Marian J Killip[2], Juan C Oliveros[3], Víctor Asensio[4], José A. Bengoechea[4], Richard E Randall[2], Juan Ortín*[1]

(1) Departamento de Biología Molecular y Celular. Centro Nacional de Biotecnología – CSIC.Madrid (2) School of Biology, Centre for Biomolecular Sciences. University of St Andrews. Scotland, UK (3) Unidad de genómica. Centro Nacional de Biotecnología – CSIC. Madrid (4) Laboratorio Patogénesis Microbiana. Fundació d'Investigació Sanitària de les Illes Balears (FISIB) Bunyola, Mallorca.

The influenza A viruses counteract the cell innate immune response at several steps. Most of the influenza virus modulation of the IFN response is provided by the multifunctional NS1 protein. In an attempt to determine whether other viral genes are also important in the interplay between the virus and the host IFN response we have undertaken a non-biassed genetic approach. We carried out serial passage of wt virus in IFN non-responsive cells and selected for viruses that were able to induce IFN. We reasoned that, by replication in the absence of the IFN selection pressure the virus could mutate at positions normally restricted and could find new optimal sequence solutions. Deep sequencing of selected virus populations and individual virus mutants indicated that non-synonymous mutations occurred at many phylogenetically conserved positions in all virus segments. Several of these mutations are recurrent in independently evolved virus populations and also in individual mutants. Of the 6 individual virus mutants studied, only one contained a mutation in NS1, althought all of them induced IFN and ISGs and were unable to counteract added IFN. Several virus mutants accumulated large amounts of defective-interfering (DI) particles but nonetheless replicated to high titres and showed mutations in the M1/M2 proteins. This result suggests that these viruses can override the NS1-mediated IFN modulation by overproduction of IFN inducers. Altogether, the results presented suggest that influenza viruses replicating in normal cells have tuned their complete genomes to survive the cellular innate immune system and serial replication in IFN non-responsive cells has allowed the virus to find new genome consensus sequences within its sequence space.

Keywords: Influenza, IFN, virus evolution, serial passages.

O-41

Spatio-temporal reconstruction of HIV-1B migration patterns in The Caribbean: a phylogenetic story

Israel Pagán*(1), África Holguín(2)

(1) Centro de Biotecnología y Genómica de Plantas (UPM-INIA) y ETSI Agrónomos. Universidad Politécnica de Madrid. Pozuelo de Alarcón, Madrid (2) Department of Microbiology. Hospital Universitario Ramón y Cajal. Madrid.

The Caribbean is one of the regions with highest HIV-1 subtype B (HIV-1B) prevalence worldwide, with several countries reaching over 2% of the population infected. Despite of this high virus burden, little is known about the timing and the migration patterns of HIV-1B in this region.

Migration is one of the major processes shaping the genetic structure and the evolutionary potential of virus populations. Thus, reconstructing the epidemiological network in The Caribbean may contribute to understand which factors determine the HIV-1B evolutionary dynamics. To address this subject, we have investigated the temporal and spatial dynamics of the HIV-1B epidemic in The Caribbean using 786 HIV-1B partial *pol* sequences from 13 Caribbean countries and a Bayesian Coalescent approach. Timing of HIV-1B introduction and virus evolutionary rates, the spatial genetic structure of the HIV-1B populations and the virus migration patterns were inferred. Results revealed that in The Caribbean HIV-1B could have been introduced in the 60s, but with most of the variability generated since the 80s. At odds with previous data suggesting that only Haiti was the origin of the epidemic in The Caribbean, our reconstruction indicated that the virus could have been also disseminated from Puerto Rico and Antigua. These two countries connected two distinguishable migration areas corresponding to the (mainly Spanish-colonized) Easter and (mainly British-colonized) Western islands, which indicates that virus migration patterns are determined by geographical barriers and by the movement of human populations among culturally related countries. The HIV-1B population was significantly structured according to the country of origin, and the genetic diversity in each country was associated with the virus prevalence, which suggests that populations evolve mainly through genetic drift. Thus, our work contributes to the understanding of HIV-1B evolution and dispersion pattern in the Americas, and its relationship with the geography of the area and the movements of human populations.

Keywords: HIV-1B phylogeography, migration patterns; population evolutionary dynamics.

SESIÓN PARALELA V

Hepatitis viruses

CHAIRS:
Antonio Mas
Rosa M. Pintó

0-42

Fast growing populations of hepatitis A virus selected from a process of cooperation / competition / recombination between populations adapted to low cellular shut-off and populations adapted to high cellular shut-off

Francisco J. Pérez Rodríguez*[1], Lucía D'Andrea[1], Montserrat de Castellarnau[1], Albert Bosch[1], Rosa M. Pintó[1]

(1) Department of Microbiology. University of Barcelona.

Hepatitis A virus (HAV) shows a highly deoptimized codon usage, a very inefficient IRES and the inability to inhibit cellular protein synthesis, thus preventing a rapid translation but ensuring a very accurate translation. This accuracy depends on the matching between newly synthesized proteins and their encoding genes, and on proper folding. Folding may depend on the pace of translating ribosomes which may be controlled by the right combination of abundant and rare codons. The process of adaptation to artificially induced cellular shut-off was driven by codon usage changes in the capsid coding region, including an initial codon deoptimization during the adaptation to low cellular shut-off followed later on by an additional codon optimization during the adaptation to high cellular shut-off. Theses dynamic codon usage changes correlated with increases and decreases of the specific infectivity respectively, which in turn correlated with changes in capsid conformation. Adaptation to cellular shut-off resulted in the selection of fast-growing populations with phenotypes much closer to that of other picornaviruses, such as poliovirus, in terms of stability and growth properties.

Competition experiments between long adapted populations to low and high cellular shut-off always resulted in the selection of even faster populations, independently of the initial proportions used. The kinetics of competition was analyzed using different genetic markers and revealed the selection of populations with several IRES mutations which could contribute to the final phenotype. Additionally, those capsid phenotypic traits critical for the specific infectivity of HAV, such as the cell binding capacity and uncoating time, of the parental populations and those of the progeny populations were compared and used as phenotypic markers to follow up the populations dynamics.

Keywords: hepatitis A, shut-off, population.

0-43

Structural analysis of HCV genomic RNA in clinical samples by means of DNA microarray technology

Ana García-Sacristán*[1,2], María Fernández-Algar[1], Antonio Madejón[2,3], José Antonio del Campo[2,4], Esteban Domingo[2,5], Jordi Gómez[2,6], Manuel Romero-Gómez[2,4], Javier García-Samaniego[2,3], Carlos Briones[1,2]

(1) Department of Molecular Evolution. Centro de Astrobiología (CSIC-INTA).Torrejón de Ardoz, Madrid (2) Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd). (3) Unidad de Hepatología. Hospital Carlos III. Madrid (4) Servicio de Medicina Interna. Hospital Universitario de Valme. Sevilla (5) Department of Virology. Centro de Biología Molecular "Severo Ochoa" (CSIC / UAM). Madrid (6) Laboratory of RNA Archaeology. Instituto de Parasitología y Biomedicina "López Neyra" (CSIC) Armilla, Granda.

Highly dynamic quasispecies variability makes it difficult to develop fully effective antiviral drugs and a vaccine against hepatitis C virus HCV (1). The search for specific sequences in HCV genome that might determine resistance or sensitivity to antiviral therapy has been inconclusive (2). In turn, one still unexplored possibility in antiviral therapy is focused on certain structural-functional elements present in the HCV RNA genome, some of which play key functional roles in viral replication, transcription and translation. In particular, the translation of HCV polyprotein is controlled by a highly structured RNA element located at the 5´ untranslated region (5'UTR) of the viral genome, termed internal ribosome entry site (IRES). Therefore, HCV IRES element has been proposed as a new target for the design of alternative antiviral drugs (3).

We have developed and optimized DNA microarrays technology to characterize the RNA secondary/tertiary structures of the HCV IRES element in clinical samples. Our fluorescence-based, antisense DNA microarrays allowed scanning the RNA accessibility of the entire IRES element in native conditions, using foot-and-mouth disease virus (FMDV) (4,5) and HCV (6,7) as the model systems. The structural information obtained is highly concordant with that derived from traditional techniques for RNA structural characterization. We are currently applying this high-throughput methodology to the analysis of the accessibility and structural stability along the HCV IRES element in clinical samples obtained from naïve and pretreated HCV-infected patients.

1. Schlütter (2010) Nature 474: S5.
2. Jardim AC et al. (2009) Infect. Genet. Evol. 9: 689
3. Wang Q et al. (2005) Molecular Therapy 12: 562.
4. Fernández N et al. (2011) Virology 409: 251.
5. Fernández N et al. (2011) Nucleic Acids Res. 39: 8572
6. Martell M et al. (2004) Nucleic Acids Res. 32: e90
7. Romero-López C et al. (2012) Nucl. Acids. Res. 40: 11697

Keywords: Hepatitis C virus (HCV), Internal ribosome entry site (IRES), viral RNA structure, DNA microarrays.

O-44

Three-dimensional visualization of forming hepatitis C Virus-like particles by electron-tomography

Nicola GA Abrescia*[(1)], Daniel Badia Martinez[(1)], Bibiana Peralta[(1)], Germán Andrés[(2)], Milagros Guerra[(2)], David Gil Carton[(1)]

(1) Structural Biology Unit. CICbioGUNE, CIBERehd. Derio, Vizcaya (2) Unidad de Microscopia Electrónica. Centro de Biología Molecular Severo Ochoa, CSIC-UAM. Cantoblanco, Madrid.

Hepatitis C virus (HCV) infects almost 170 million people per year being one of the major causes for chronic liver disease. As other flaviviruses, HCV is thought to replicate in the cytoplasm acquiring the viral envelope by budding through the endoplasmatic reticulum (ER) but its assembly pathway with the involvement of lipid-droplets, architecture and structures of its envelope proteins are poorly understood.

With this paucity of three-dimensional (3D) structural information, applying a reductionist and mechanistic approach we embarked in studying HC virus-like particles produced in insect cells. Using electron tomography of plastic-embedded sections of Sf9 cells, we have provided a 3D morphological description of these HCV-LPs at the ER site as surrogate of *wt*-HCV allowing to view the particles one-by-one and each in its budding stage (differently to the previously used 2D imaging technique that displays the HCV-LPs as projection and whose shape doesn't necessarily reflect the budding stage). Tomographic data were collected on our JEOL JEM2200-FS microscope on a 4Kx4K CCD camera. Tomograms were processed with IMOD, denoised using Tomobflow and analysed with Chimera and Amira softwares.

Our data provide a 3D sketch of viral assembly at the ER site with different budding stages identified as three main classes: (i) membrane areas of protein concentration, (ii) cup-shaped particles and (iii) particles on the verge of scission. Furthermore we could detect proximity of buds from which we hypothesize a mechanism of large particles formation.

Acknowledgments: We are extremely grateful to Genentech and S. Foung for providing respectively the AP33 antibody and the antibodies CBH-2, -5 and -7 against glycoprotein E2.

Keywords: hepatitis C virus, HC-virus-like-particles, cellular tomography.

O-45

Ultra-deep pyrosequencing to study hepatitis C virus superinfection after liver transplantation and HBV complexity under antiviral treatment

Josep Gregori i Font*[1,2], Sofia Pérez del Pulgar[1], Francisco Rodríguez Frías[3], Patricia Gonzalez[1], Damir García Cehic[4], María Homs[3], David Tabernero[3], Santseharay Ramírez[1,5], María Cubero[2,4], Juan I Esteban[1], Xavier Forns[1], Josep Quer[4]

(1) Liver Unit. Laboratori Malalties Hepàtiques. Lab 006. VHIR HUVH. Barcelona (2) Business Development. Roche Diagnostics SL. Sant Cugat del Vallès. Barcelona (3) Biochemistry Department. VHIR.HUVH. CIBERehd. Barcelona (4) Liver Unit, Lab. Malalties Hepatiques. VHIR.HUVH. CIBERehd. Barcelona (5) Department of Infectious Diseases and Clinical Research Centre. Copenhagen Hepatitis C Program. Copenhagen. Denmark.

Backgroundand aims: Superinfection occurs when a chronic HCV patient is transplanted with an infected graft. Quasispecies complexity evolution in chronic Hepatitis B may have a role in response to Lamivudine (LMV).

Methods: 6 HCV-infected patients who underwent LT with HCV-infected grafts. Samples: Donor (D), Recipient (D) and day1, d2, week 1, months 1, 4, 6 and 12. 30 samples from 10 CHBV patients pre-, during, at final of treatment. 18 samples from 9 patients treated with Lamivudine (pre and breakthrough -VBK). Ultra-deep pyrosequencing (UDPS) 454 GS-FLX platform.

Results: Successive expansions and contractions of quasispecies were observed, evolving in all cases towards a more homogeneous population, with a relatively low genetic variability. In transplanted patients the most complex viral population (D or R) excluded the other and became dominant. Interestingly, in case 2, viral coexistence lasted even after the first year after LT.

HBV complexity mainly increased during not treatment period and decreased in 80% of VBK at PC/C region after LMV treatment. In contrast the RT region, variability increased in 80% of HBeAg+ve patients, but decreased in 75% of HBeAg-ve. Variability in S polymorphic positions did not always coincided with immune escape positions. Some unexpected substitutions rtA200V and rtT184S in the overlapping RT region, showed significant increase at VBK in 22% and 3.7% of cases, respectively, and rtV163I variant was selected or increased during treatment.

Conclusions: Our results show that during superinfection with a different HCV strain in the LT, the viral population with the highest diversity always outcompetes the other and becomes dominant. The exclusion of non-dominant can take place as early as the first day or after several months following LT. However, the excluded virus may remain as a minority population (even after 1 year) and could emerge if there were any changes in the environment.

The decrease after VBK of LMV treatment of HBV quasispecies complexity in PC/C region seems indicate the selection of some specific antiviral resistant variants. However the behaviour of quasispecies seems different in RT/S region which de decrease in the HBV genetic variability after LMV depends on the HBeAg status, which in turn could be related to the different anti-HBeAg response of these patients to LMV. Interestingly, escape mutations in the "a determinant" are rarely observed.

Keywords: UDPS, HCV, HBV, evolution, quasispecies, complexity.

O-46

Lethal mutagenesis is involved in hepatitis C virus extinction by ribavirin in cell culture

Ana Mª Ortega Prieto*[1], Julie Sheldon[1], Ana Grande Pérez[2], Héctor Tejero[1,3], Josep Gregori[4,5], Josep Quer[4,6], Juan I Esteban[4,6], Esteban Domingo[1,6], Celia Perales[1,6]

(1) Departamento de Virología y Microbiología. Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM).Cantoblanco, Madrid (2) Departamento de Biología Celular, Genética y Fisiología. Facultad de Ciencias, Universidad de Málaga. (3) Departamento de Bioquímica y Biología Molecular Universidad Complutense de Madrid. (4) Liver Unit, Internal Medicine. Lab. Malalties Hepàtiques. Vall D'Hebron Institut Recerca Hospital (VHIR-HUVH). Barcelona. (5) Roche Diagnostics, S.L. Sant Cugat del Vallès. Barcelona (6) Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd).

Hepatitis C virus (HCV) infections affect about 180 million people worldwide, and about 75% of newly infected patients progress towards a chronic infection, with a risk of severe liver disease. HCV is a hepacivirus of the*Flaviviridae* family that displays the error-prone replication and quasispecies dynamics typical of RNA viruses. The current standard of care treatment consists of the combination of pegylated-interferon-a (IFN-a) and the purine nucleoside analogue ribavirin (1-*b*-D-ribofuranosyl-1-*H*-1,2,4-triazole-3-carboxamide) (Rib) (IFN-a+Rib). Rib is a recognised mutagenic agent for several other RNA viruses, but it is not clear whether Rib exerts its anti-HCV activity through mutagenesis or other mechanisms. Elucidation of the anti-HCV mechanism of Rib is highly relevant because recent studies have shown that when a mutagenic agent is a component of therapy, a sequential inhibitor-mutagen administration can have an advantage over the corresponding combination (Perales et al. 2012).

Here we describe the use of HCV rescued following transcription of plasmid Jc1FLAG (p7-nsGluc2A) (genotype 2a) (abbreviated as HCVp0) to document mutagenic activity of Rib on HCV during replication in human hepatoma cells. Multiple virus passages in Huh-7.5 cells were carried out in the absence or presence of different Rib concentrations. A mutagenic activity of Rib was documented by statistically significant increases of mutant spectrum complexity (as determinated by mutation frequencies, genetic distances and Shannon entropy), and a mutational bias in favour of G®A and C®U transitions using molecular cloning and Sanger sequencing as well as ultra-deep pyrosequencing. Rib treatment resulted in imbalance of the NTP pools (a reduction of intracellular GTP and an increase of UTP, ATP and CTP), control experiments using mycophenolic acid and guanosine indicated that GTP depletion could not explain Rib mutagenesis. Moreover, HCV extinction by Rib, but not by the non-mutagenic HCV inhibitor mycophenolic acid, occurred with decreases of specific infectivity, a feature typical of lethal mutagenesis. Thus, at least part of the antiviral activity of Rib on HCV in cell culture is exerted via lethal mutagenesis. This result opens the way to model studies on the efficacy of lethal mutagenesis-based treatments for HCV infections.

-Perales C., Iranzo J., Manrubia S.C., Domingo E. 2012.The impact of quasispecies dynamics on the use of therapeutics. Trends in Microbiol. : 595–603.

Keywords: ribavirin, hepatitis C, lethal mutagenesis.

O-47

Molecular epidemiology of viral hepatitis in Spain

Ana Avellón[1]

(1) Department of Hepatitis Unit. National Centre of Microbiology.(ISCIII) Majadahonda, Madrid.

Hepatitis A: HAV infection in Spain has an incidence of 2.23*. in approximately 40% of HAV outbreaks the source of infection cannot be identified (only 4 of 74 in Spain, 2010).Infection affected mainly to men (59.6%) less than 9 years. In Spain few restricted studies have been made involving mostly G-IB. Molecular epidemiology performed over acute cases received at the National Centre of Microbiology (NCM) from different regions of Spain since 2000 showed that G-IA is the most frequent detected but G-IB also circulates. Additionally our analysis shows clustering sequences associated to outbreaks both in G-IA and G-IB.

Hepatitis B: HBV had an incidence in Spain of 1.86*, affecting mostly to men (70.2%) aged 25 to 34. Molecular epidemiology can be performed to prove the phylogenetic connection of HBV cases if a transmission is suspected. We present the study of 13 cases on infection involved in an outbreak in a retirement home.

Hepatitis C: Regarding HCV only Microbiology Reporting System data (335 cases in 2008) are available. In the NCM we study all the request of phylogenetic analysis of cases, in which association of HCV infection to medical procedures is suspected. Here we present the analysis of 3 of these cases.

Hepatitis D: The prevalence of HDV infection varies according to the geographical area being estimated around 15 million HDV carriers worldwide. In Spain there are no data of incidence and neither of molecular epidemiology of HDV. NCM reception of samples for HDV screening has been increasing since 2007, just like positive cases detected. A total of 64 RNA extracts from HDV PCR positive samples received were included in the first wide HDV molecular epidemiology study performed in Spain. G-I revealed to be the most prevalent in Spain. G-I presents high diversity with clustering that may reflect the complex epidemiology distribution of HDV.

Hepatitis E: HEV G-3 is causing acute hepatitis in Spain and has been detected in swine, wild boar and in sewage. NCM is receiving an increasing number of samples for HEV diagnosis, and has collected about 40 HEV RNA positive samples until 2012. The preliminary results of molecular epidemiology indicate that a variety of sub-genotypes might be circulating in Spain (3f the most prevalent), including one for which it seems not to be available sequences for comparison. It is interesting to go in depth in the comparative study of source and clinical cases sequences. * c/100.000, EDO system, 2010, NCE

Keywords: hepatitis virus, HAV, HBV, HCV, HDV, HEV.

SESIÓN PARALELA VI

Virus entry and exit, and mechanisms of viral cell-to-cell transmission

CHAIRS:
José María Almendral
Julià Blanco

0-48

Clathrin mediates hepatitis C virus egress but not aplipoprotein B and E secretion

Virgínia M. Gondar*[1], Ignacio B. Español[1], Francisca M. Jiménez[1], Marisa G. Buey[2], Pablo G. Gastaminza[3], Pedro L. Majano[1]

(1) Unidad de Biología Molecular. Instituto de Investigación Biomédica Hospital La Princesa. Madrid (2) Unidad de Hígado. Instituto de Investigación Biomédica Hospital La Princesa. Madrid (3) Departamento de Biología Molecular y Celular. Centro Nacional de Biotecnología.(CSIC). Madrid.

Hepatitis C virus (HCV) entry into hepatocytes relies on clathrin-mediated endocytosis. However, the possible role of clathrin in other steps of the viral cycle remains unexplored. Herein, we showed that clathrin and clathrin adaptor AP-1 depletion reduced HCV secretion from JFH-1-infected Huh7 cells, in terms of both extracellular HCV RNA levels and infective viral particles. The same effect was observed after treatment with the dynamin inhibitor dynasore and the clathrin inhibitor pitstop-2. In addition, both inhibitors impaired HCV RNA release from 3D cultured, hepatocyte-like polarized cells. Of note, intracellular HCV RNA levels were not reduced by clathrin depletion or dynamin inhibition. Since it has been proposed that HCV exits the cell in association with very low-density lipoproteins (VLDL), we studied whether the exocytosis of the VLDL components apolipoprotein B (apoB) and E (apoE) was altered after clathrin knockdown. We observed that whereas apoB and apoE were necessary for HCV assembly, their secretion was clathrin independent. Though, both brefeldin A (BFA) and dynasore treatment blocked HCV RNA, apoB and apoE secretion. Finally, detergent-free immunoprecipitation experiments showed that intracellular apoE-associated viral RNA levels increased in the presence of BFA and that intracellular infectivity of BFA-treated cells was reduced after apoE immunoprecipitation and partially blocked by adding an anti-apoE antibody during titration, suggesting an apoE-HCV association in the endoplasmic reticulum. In summary, we showed that although HCV assembly depended on apoB and apoE, HCV and VLDL might exit cells through clathrin dependent or independent routes, respectively.

Keywords: HCV, clathrin, apolipoproteins, VLDL, assembly, egress.

0-49

Role of the small GTPase Rab27a during herpes simplex virus infection of oligodendrocytic cells

Antonio J. Crespillo*[1], Raquel Bello Morales[1], Alberto Fraile Ramos[2], Antonio Alcina[3], Enrique Tabarés[4], José Antonio López Guerrero[1]

(1) Departametno de Biología Molecular. CBM-UAM-CSIC. Cantoblanco, Madrid (2) Facultad de Medicina. Universidad Complutense. Madrid (3) Instituto de Parasitologia y Biomedicina "Lopez-Neyra".Armilla, Granada (4) Facultad de Medicina. UAM. Madrid.

Background: The morphogenesis of herpes simplex virus type 1 (HSV-1) comprises several events, of which some are not completely understood. It has been shown that HSV-1 glycoproteins accumulate in the trans-Golgi network (TGN) and in TGN-derived vesicles. It is also accepted that HSV-1 acquires its final morphology through a secondary envelopment by budding into TGN-derived vesicles coated with viral glycoproteins and tegument proteins. Nevertheless, several aspects of this process remain elusive. The small GTPase Rab27a has been implicated in regulated exocytosis, and it seems to play a key role in certain membrane trafficking events. Rab27a also seems to be required for human cytomegalovirus assembly. However, despite the involvement of various Rab GTPases in HSV-1 envelopment, there is, to date, no data reported on the role of Rab27a in HSV-1 infection.

Results: Herein, we show that Rab27a colocalized with GHSV-UL46, a tegument-tagged green fluorescent protein-HSV-1, in the TGN. In fact, this small GTPase colocalized with viral glycoproteins gH and gD in that compartment. Functional analysis through Rab27a depletion showed a significant decrease in the number of infected cells and viral production in Rab27a-silenced cells.

Conclusions: Altogether, our results indicate that Rab27a plays an important role in HSV-1 infection of oligodendrocytic cells.

Keywords: HSV-1, oligodendrocytes, Rab27a, viral egress, morphogenesis, tegument.

0-50

Alphaviruses can propagate in the absence of capsid protein

Marta Ruiz Guillen[1], Evgeni Gabev[1], José I. Quetglas[1], Alejandro Aranda[1], Erkuden Casales[1], Jaione Bezunartea[1], Marina Ondiviela[2], Jesús Prieto[1], Nicola Abrescia[2,3], Cristian Smerdou*[1]

(1) Departamento de Terapia Génica y Hepatología. CIMA. Pamplona (2) Structural Biology Unit. CIC bioGUNE. Derio, Vizcaya (3) IKERBASQUE. Basque Foundation for Science. Bilbao.

Alphaviruses are enveloped viruses that contain a positive-strand RNA genome packaged into a nucleocapsid of icosahedral symmetry. For most viruses, the capsid is an essential protein layer that encloses and protects the viral genome. The budding of alphaviruses is driven by specific interactions between nucleocapsids assembled in the cytoplasm and the viral envelope proteins present at the plasma membrane (E1 and E2). It has been previously demonstrated that expression of capsid and envelope proteins in infected cells is an absolute requirement for alphavirus budding and propagation. In contrast to these data, in the present study we show that Semliki Forest virus (SFV) and Sindbis virus (SIN) genomic RNAs lacking the capsid gene can propagate efficiently in both mammalian and mosquito cells. The small membrane 6K protein was also dispensable for propagation, at least for SFV. E1 and E2 proteins seemed to be required for this process, since a neutralizing antiserum against these proteins was able to block

propagation in cell culture. In addition, overexpression of E1 and E2 proteins was able to increase the propagation rate. Propagation was not mediated by fusion of infected with non-infected cells, and cell-to-cell contacts did not seem to be essential for this process. Instead, cells infected or transfected with SFV devoid of capsid were able to release infectious microvesicles which contained envelope proteins at their surface and viral RNA inside. These microvesicles appeared to infect cells following a process that also required an acidic pH in endosomes. In order to evaluate the possibility of using these infectious membranous particles as expression vectors, the GFP gene was cloned into the SFV genome devoid of capsid downstream of a second subgenomic promoter. This vector was able to transfer GFP expression from initially infected or transfected cells to most cells in a culture after a few hours. Although these microvesicles were produced at low levels, this minimalist infectious system could impact the way we see viruses today. In fact, they could mimic some sort of primordial envelope viral systems that had not yet acquired a nucleocapsid able to efficiently package and deliver the viral genome.

Keywords: alphavirus, capsid, envelope proteins, budding.

O-51

Role of the 5' untranslated region of the Alfalfa mosaic virus RNA 3 in cell-to-cell and long distance transport

Thor Fajardo[2], Ana Peiró[1], Vicente Pallás[1], Jesús A. Sánchez Navarro*[1]

(1) Departamento de Biología del Estrés. Instituto de Biología Molecular y Celular de Plantas (CSIC-UPV).Valencia (2) Departamento de Virología. Embrapa Uva e Vinho . Bento Gonçalves. Brasil.

After the start of the infection in a single cell, plant viruses need to invade the adjacent cells, a process denominated cell-to-cell transport, as a previous step to invade the distal parts of the host through the vascular system or systemic transport. The capacity to reach the uninoculated parts of a plant implies that the virus should infect specific cells located at the vascular tissue. In most cases, virus particles are required for this vascular transport. In the present study we have addressed the characterization of viral determinants critical for the long distance transport using the *Alfalfa mosaic virus* (AMV) model system, which requires virus particle for the systemic transport.

AMV is the type member of the Alfamovirus genus within the family *Bromoviridae*. Its genome consists of three positive RNAs. Monocistronic RNAs 1 and 2 encode P1 and P2 proteins of the RNA polymerase complex, respectively. RNA 3 contains two open reading frames encoding the movement protein (MP) and coat protein (CP), which is expressed through a subgenomic RNA or RNA 4.

Previous analysis showed that the AMV MP gene is functionally interchangeable for long and short distance transport by the corresponding gene of viruses belonging to eight genera of the viral family 30K (Sanchez-Navarro et al.; 2006 Virology 341: 66-73; Sánchez-Navarro et al., 2010, J. Virology 84: 4109-4112). However, the exchange of the *Brome mosaic virus* MP lacking the C-terminal 48 amino acid residues, generated a chimeric RNA 3 (MPBMV255/CP) defective for long-distance transport (Sánchez-Navarro et al., 2001; MPMI 14: 1051-1062). In this study we performed viral evolution experiments, addressed to characterize RNA 3 determinants of the chimera MPBMV255 / CP critical for systemic transport. After the seventh passage, we observed systemic infection in all lines. The analysis of the nucleotide sequence revealed that all RNA 3 variants present in the upper parts of the plants contained deletions at the 5' untranslated region (5' UTR). Further analysis of the evolved 5'UTR revealed that this region, in spite of reducing the expression of the MP and drastically the encapsidation of the viral progeny, incremented the cell-to-cell transport. Interestingly, we observed that the modified 5'UTR permits the systemic transport of an AMV variant defective in virus particles formation. The evolutive implications of these observations for the cell-to-cell and systemic transport of plant viruses will be discussed.

Keywords: Systemic transport; 30K family; Alfamovirus; virus evolution.

0-52

Structural studies of PRD1 genome delivery device

Bibiana Peralta[1], David Gil-Carton[1], Daniel Castaño Díez[3], Aurelie Bertin[4], Claire Boulogne[4], Hanna M. Oksanen[5], Dennis H. Bamford[5], Nicola G.A. Abrescia*[1,2]

(1) Structural Biology Unit. CICbioGUNE, Derio, Vizcaya (2) IKERBASQUE. Basque Foundation for Science. Bilbao (3) Center for Cellular Imaging and Nano-Analitics (C-CINA) Biozentrum. University of Basel. Switzerland (4) Institut de Biochimie et Biophysique Moléculaire et Cellulaire. Université de Paris-Sud. France (5) Institute of Biotechnology and Department of Biosciences. University of Helsinki. Finland.

PRD1 is an internal membrane-containing bacteriophage that infects Gram-negative cells. A wealth of biochemical and structural information has been accumulated on PRD1 during the past 20 years. So far, it remains the only phage with a membrane whose icosahedral structure has have been visualized at 4.2Å by X-ray crystallography elucidating fundamental aspects of viral evolution. To deliver its double-stranded DNA, the icosahedral protein-rich virus membrane transforms into a tubular structure protruding from one of the twelve vertices of the capsid.

Here, using a combination of electron microscopy techniques, we study PRD1, the best understood model for lipid-containing viruses, to unveil the mechanism behind the genome translocation across the cell envelope.

We show that this viral nanotube exits from the same unique vertex used for DNA packaging and crosses the capsid through an aperture that correspond to the loss of the peripentonal P3 major capsid protein trimers, penton protein P31 and membrane associate protein P16. Changes in osmolarity and loss of capsid-vesicle interactions at the de-capping vertex nucleate the remodeling of the internal viral membrane allowing the polymerization of the tail tube structured by membrane-associated proteins. We also visualize this proteo-lipidic tube *in vivo*, piercing the gram-negative bacterial cell envelope and shuttling its genomic cargo.

Keywords: viral genome delivery, viral nanotube, membrane, electron microscopy, cryo-electron tomography.

0-53

Anti-HIV activity of thiol-ene carbosilane dendrimers and potential topical microbicide

Javier Sánchez Rodríguez*[1,2], Marta Galán[3], Daniel Sepúlveda Crespo[1,2], Rafael Gómez[3], Francisco J. de la Mata[3], José L. Jiménez[1,2], M. Ángeles Muñoz Fernández[1,2]

(1) Departamento de Inmunobiología Molecular. Hospital General Universitario Gregorio Marañón. Madrid (2) Plataforma de Laboratorio. Hospital General Universitario Gregorio Marañón. Madrid(3) Departamento de Química Inorgánica. Universidad de Alcalá de Henares. Madrid.

Background. The concept of "microbicide" has been born out of the unavailability of a vaccine against HIV and the problems of women to negotiate the use of preventive prophylaxis by their partners. The polyanionic carbosilane dendrimers approach should be taken into consideration when designing new microbicide.

Objective. To design the synthesis of G2-STE16 and G2-CTE16 carbosilane dendrimers and to research as topical microbicide against HIV-1

Results. G2-STE16 and G2-CTE16 have shown high biosafety in human epithelial cell lines derived from uterus and vagina and in primary human peripheral blood mononuclear cells (PBMC). The G2-STE16 and G2-CTE16 dendrimers have a greater capacity to block the entry of different X4 and R5 HIV-1 isolates inside epithelial cells. Both dendrimers protect the epithelial monolayer cells from the disruption and prevent the HIV-1 infection of activated PBMC. The treatment of uterus and vagina epithelial cells with G2-STE16 and G2-CTE16 did not produce changes in activation and proliferation of PBMC. These dendrimers did not cause changes in the expression of CD4, CCR5 or CXCR4 in PBMC. Interestingly enough, our dendrimers did not affect vaginal microbiote and sperm survival.

Methods. We used the HEC-1A and VK2/E6E7 lines of the female genital tract at first cellular barrier against HIV infection and peripheral blood mononuclear cells (PBMC). Inhibition of HIV replication and internalization were done to study anti-HIV activity

in epithelial cells. Assays were performed by flow cytometry to determine cell activation, proliferation and cytokines profile.

Conclusions. G2-STE16 and G2-CTE16 are easy of synthesize, are compatible with the functional groups and the purification steps are easy and short. Our results have clearly demonstrated that these dendrimers have high potency as topical microbicide against HIV-1.

Keywords: microbicide, HIV, dendrimer, biosafety.

0-54

Cancer virotherapy and adenovirus

Ramón Alemany[1]

(1) Translational Research Laboratory. Institute of Biomedical Reasech of Bellvitge (IDIBELL). Catalan Institute of Oncology. L'Hospitalet de Llobregrat. Barcelona.

Cancer virotherapy has advanced to clinical trials as an immunotherapy strategy. Among different oncolytic viruses, highly selective adenoviruses have been designed with transcriptional control elements, deletions of viral functions, and targeting tumor-associated receptors. Although well tolerated, efficacy has been limited by a poor systemic tumor targeting and intratumoral spread. Further, an anti-viral immune response often clears the virus without eliciting an anti-tumor response. Modification of the virus capsid, combination with cellular vehicles, and expression of transgenes can help to solve these limitations. ICOVIR17K (VCN-01) is an oncolytic adenovirus that has a modified capsid to increase tumor targeting and expresses hyaluronidase to increase its intratumoral spread. This virus is proposed for clinical development.

Keywords: virotherapy, cancer, oncolytic adenovirus.

SESIÓN PARALELA VII

Virus-host interactions and genome-wide association studies

CHAIRS:
Juana Díez
Fernando García-Arenal

O-55

Influenza A virus NS1 and PI3K: strain and isotype specificity of a complex virus-host interaction

Juan Ayllón Barasoain*[1], Benjamin G. Hale[2], M. Teresa Sánchez Aparicio[1], Adolfo García Sastre[1]

(1) Department of Microbiology. Icahn School of Medicine at Mount Sinai. New York, USA (2) Centre for Virus Research. MRC-University of Glasgow. UK.

The NS1 protein of influenza A viruses is a multifunctional virulence factor with a broad array of interactors within the infected cell. Amongst them, NS1 is known to bind and activate the host class IA phosphoinositide-3-kinase (PI3K), a critical regulatory node in multiple cell signaling networks that influences cell physiology at various stages including cell growth, survival, trafficking and immune function. The biological purpose of the activation of PI3K by NS1 remains, however, unclear. Here, we show how this activation contributes to the virus replication and virulence *in vivo* and how this relevance is viral strain specific despite all the NS1 tested being equally able to activate PI3K. Furthermore, we have found that there is an additional layer of specificity within the host factor itself: class I PI3K are obligate heterodimeric enzymes composed of a regulatory, inhibitory subunit (mainly p85 or p85) and a catalytic subunit, p110, with three isotypes designated , and . NS1 is known to specifically bind and repress the inhibition caused by p85 . Here, we show that NS1 differentially redistributes and activates heterotypic PI3K complexes depending on their catalytic subunit isotype. We postulate that different NS1 strains induce heterotypic PI3K complexes to signal from distinctive platforms and through different pathways, thus affecting overall viral fitness in varying degrees. Our findings suggest that activation of PI3K by influenza A virus NS1 has been diversely shaped through evolution in distinct viral strains to take advantage of the variability within PI3K signaling, providing a challenging example of a complex and multi-variant virus-host interaction.

Keywords: Influenza virus, NS1, PI3K, virulence, BiFC, signaling.

O-56

Role of the cellular phosphatase DUSP1 in vaccinia virus infection

Ana Cáceres*[1], Beatriz Perdiguero[1], Carmen Elena Gómez[1], María Victoria Cepeda[1], Carlos Óscar Sorzano[2], Carme Caelles[3], Mariano Esteban[1]

(1) Departamento de Biología Molecular y Celular. Centro Nacional de Biotecnología. Madrid (2) Unidad de Biocomputación. Centro Nacional de Biotecnología. Madrid (3) Departamento de Bioquímica y Biología Molecular. Universidad de Barcelona.

Poxviruses encode a large variety of proteins that mimic, block or enhance host cell signaling path-

ways on their own benefit. It has been reported that mitogen-activated kinases (MAPKs) are specifically up-regulated during vaccinia virus (VACV) infection. In this study, we have evaluated the role of the MAPK negative regulator dual specificity phosphatase 1 (DUSP1) in the infection of VACV. The results obtained in this analysis demonstrate that DUSP1 mRNA transcription and protein expression are enhanced upon infection with wild-type (WT) virus WR or with the attenuated VACV mutants MVA and NYVAC and this up-regulation is dependent on early viral protein synthesis. In the absence of DUSP1 in cultured cells, there is an increased activation of its molecular targets JNK and ERK and an enhanced WR replication. Moreover, DUSP1 knock-out (KO) mice are more susceptible to WR infection probably as a result of the enhanced virus replication that we observed in the lungs from KO infected animals. Interestingly, MVA, which is known to perform non-permissive infections in most mammalian cell lines, is able to grow in KO immortalized murine embryo fibroblasts (MEFs). Confocal and electron microscopy assays showed that, in the absence of DUSP1, MVA morphogenesis is similar as in permissive cell lines. By the specific inhibition of MAPKs during MVA infection of KO cells, we have determined that the activation of MAPKs is at least partially responsible for the ability of MVA to replicate in the absence of DUSP1. In addition, we observed an increment in the secretion of pro-inflammatory cytokines at early times post-infection and an enhancement in the adaptive immune response when we infected KO mice by different routes. Altogether, these findings revealed that DUSP1, through the modulation of MAPKs, is involved in the replication and host range of VACV and in the regulation of host immune responses. Thus, DUSP1 is a host factor that acts as an antiviral defense mechanism against a poxvirus infection.

Keywords: MAPKs, virus-host interaction, morphogenesis, phosphatase, VACV

O-57

Regulation of PKR activity by SUMO

Carlos F. de la Cruz Herrera*[1], Michela Campagna[1], Laura Marcos Villar[1], María A. García[2], Valerie Lang[3], Anxo Vidal[4], Manuel S. Rodríguez[3], Mariano Esteban[1], Carmen Rivas[1]

(1) Departamento de Biología Molecular y Celular. Centro Nacional de Biotecnología CSIC. Madrid (2) Unidad de Investigación. Hospital Universitario Virgen de las Nieves. Granada (3) Department of Ubiquitylation and Cancer Molecular Biology laboratory. Inbiomed. San Sebastián (4) Departamento de Fisioloxía and Centro de Investigación en Medicina Molecular (CIMUS). Universidade de Santiago de Compostela.

The double-stranded RNA (dsRNA)-dependent kinase (PKR) is an interferon-inducible serine/threonine kinase that has been linked to normal cell growth and differentiation, inflammation, cytokine signalling and apoptosis. The main function attributed to PKR is the ability to phosphorylate

the alpha-subunit of the eukaryotic initiation factor (eIF)-2 complex that results in a shut-off of general translation and the inhibition of virus replication. PKR is required for interferon a/b production in response to a subset of RNA viruses and plays also a major role in the activation and/or regulation of several transcription factors such as nuclear factor (NF)-kB and p53. PKR is activated in response to diverse stress signals such as the presence of dsRNA, cytotoxic cytokines, growth factor deprivation, oxidative stress, and DNA damage. Activation of PKR results from homodimerization followed by autophosphorylation, and it can be modulated positively by the interaction with cellular proteins such as TAR RNA binding protein (TRBP), MDA-7/interleukin-24, ISG15 and PKX activator/PKR associated protein X (PACT/RAX), or negatively by cep58IPK, nucleophosmin and heat shock proteins 90 and 70. Here we analyzed the interaction of PKR with the small ubiquitin-like modifiers (SUMO) 1 and 2 and evaluated the consequences of PKR SUMOylation in the context of viral infection. Our results indicate that PKR is modified by SUMO1 and 2, *in vitro* and *in vivo*. We also show that SUMO modification enhances PKR activation in response to different stimuli and activates the protein even in the absence of virus infection. Furthermore, a mutant of PKR that cannot be SUMO conjugated is partially impaired in its ability to inhibit protein synthesis. These data suggest a novel pathway mediating the activation of PKR and the inhibition of virus replication.

Keywords: PKR, SUMO, antiviral acitivity.

0-58

Regulation of SAMHD1-mediated HIV-1 restriction by cytokines IL-12 and IL-18

Eduardo Pauls[1], Esther Jiménez[1], Margarida Bofill[1], José A. Esté*[1]

[1] Fundació irsiCaixa. Hospital Germans Trias i Pujol. Universitat Autónoma de Barcelona.

SAMHD1 (sterile alpha motif and histidine/aspartic acid (HD) domain-containing protein 1) has been identified as a novel HIV-1 restriction factor in myeloid cells and resting CD4+ T lymphocytes. SAMHD1 regulates the pool of intracellular nucleotides to control HIV replication. However, how the expression and function of SAMHD1 are regulated the expression and function of SAMHD1 is unknown. Macrophage subsets polarize depending on the environment and the activation signal they are submitted to. We have found that monocyte-derived macrophages (MDM) differentiated with a combination of interleukins-12 and -18 (IL12/IL18) led to HIV-1 resistant macrophages in contrast to macrophages differentiated in the presence of M-CSF. In addition, M-CSF-derived macrophages treated with IL12/IL18 became resistant to HIV-1 infection. The HIV-1 restriction factor SAMHD1 was significantly overexpressed in IL12/IL18 MDM compared to M-CSF MDM. Interestingly, SAMHD1 overexpression induced by IL12/IL18 was not dependent on interferon-g. Degradation of SAMHD1 by RNA interference or viral-like particles carrying the lentiviral protein Vpx restored HIV-1 infectivity of IL12/IL18 MDM,

demonstrating that IL12/IL18-mediated HIV-1 restriction was due to SAMHD1. Inflammatory cytokines such as IL-12 or IL-18 may contribute to the response against HIV-1 infection through the induction of SAMHD1.

Keywords: SAMHD1, interferon, cytokines, macrophages.

O-59

Specific residues of PB2 and PA influenza virus polymerase subunits confer the ability for RNA polymerase II degradation and increase the virus pathogenicity in mice

Amelia Nieto*[1], Catalina LLompart[1], Ariel Rodríguez[1]

(1) Departamento de Biología Molecular y Celular. Centro Nacional de Biotecnologia-CSIC, Madrid. (2) Ciber de Enfermedades Respiratorias.

Influenza virus employs an unusual RNA transcription mechanism that uses as primers short-capped oligonucleotides scavenged from newly synthesized RNAP II transcripts and this fact entirely decides its life cycle since it requires a functional coupling between viral and cellular transcription machineries. Despite that association, the virus induces RNAP II degradation once viral transcription is finished and cellular transcription is no longer required. This process seems to be a general feature of pathogenic strains being the viral polymerase responsible for the degradation. Viral polymerase is a heterotrimer composed by three different subunits, named PA, PB1 and PB2. Reassortants viruses from A/PR8/8/34 (PR8) strain that induce (hvPR8) or not (lvPR8) RNAP II degradation, have allowed the identification of PA and PB2 subunits as responsible for the degradation process. Three changes in PB2 sequence (I105M, N456D and I504V) and two in PA (Q193H, I550L) differentiate PA and PB2 of lvPR8 from hvPR8.

To analyze the role of these specific residues, we have rescued the corresponding single, double and triple PB2 and PA mutant viruses. Changes at positions 504 of PB2 together with 550 of PA confer the ability of lvPR8 for RNAP II degradation and conversely, these changes in hvPR8 abolish its degradation capacity. Additionally, the insertion of these mutations in the lvPR8 or the hvPR8 polymerase, gives raise to an increased or decreased viral polymerase activity, indicating that RNAP II degradation and increased polymerase activity are linked phenotypes.

We have also analyzed whether these residues also play a role in different strains. Mutations at positions 504 of PB2 and 550 of PA present in the lvPR8 sequence have been inserted in the corresponding PB2 and PA subunits of the A/Victoria/3/75 and A/California/07/09 (CAL) viruses. In both cases their changes abolish the RNAP II degradation capacity. Moreover the pathogenicity of the wild type or mutated CAL viruses has been examined in an *in vivo* model. Loss of body weight was more pronounced in the wt CAL-infected mice and 75%

lethality was observed in this situation compared with 100% survival on mutant CAL- or mock-infected animals. These results confirm that the introduction of PB2 and PA residues that abolish the RNAP II degradation in infected cells and diminish the activity of the polymerase, also decrease the pathogenicity of the virus in the murine model.

Keywords: influenza, host-interaction, pathogenesis.

O-60

The importin-α7 gene is a determinant of influenza virus cell tropism in the murine lung

Patricia Resa Infante*[1], René Thieme[2], Petra Arck[2], Rudolph Reimer[3], Gülsah Gabriel[1]

(1) Department of Influenza Pathogenesis. Heinrich-Pette-Institut; Leibniz Institute for Experimental Virology. Hamburg, Germany (2) Department of Obstetrics and Fetal Medicine. University Medical Center Hamburg-Eppendorf. Hamburg, Germany (3) Electron Microscopy and Micro-Technology . Heinrich - Pette - Institut; Leibniz Institute for Experimental Virology. Hamburg, Germany.

Influenza A viruses are a continuous threat to humans due to their ability to cross species barriers and adapt to new hosts. Since viral transcription and replication takes place in the nucleus of the host cell, the viral polymerase complex needs to adapt to the mammalian nuclear import machinery upon interspecies transmission. It has been shown that differential use of importin- isoforms governs host adaptation of influenza viruses. While growth of highly pathogenic avian influenza viruses depends on importin- 3, growth of mammalian viruses depends on importin- 7 expression.

Here, we have performed a comparative analysis of the viral replication efficiency and cell tropism in the lungs of wildtype (WT) and importin- 7-knockout ($7^{-/-}$) mice using a recombinant H1N1 influenza virus which carries a GFP reporter gene in the NS segment. We observed that upon GFP virus infection, 80% of the WT mice succumbed to infection within 8 days, while all infected $7^{/}$ mice survived. Analysis of viral lung kinetics revealed extensive infiltration and destruction of the alveolar epithelium in WT mice. In contrast, lung tissue of $7^{-/-}$ animals was mostly intact and bronchial epithelium was preferentially infected. The quantification of the cellular immune response by flow cytometry revealed significantly increased numbers of granulocytes in the lungs of infected $7^{-/-}$ mice in comparison to WT mice on day 3 post infection. While the number of macrophages and dendritic cells did not differ, histological studies displayed virus-RNA positive macrophages in the infected lungs of WT but not $7^{-/-}$ mice. This correlates with virus replication efficiency *in vitro* where higher replication rates were observed in primary macrophages of WT mice. However, we did not detect significant differences in virus clearance by primary macrophages from WT or $7^{-/-}$ mice. This suggests

that importin- 7 gene is mainly required for the virus replication not only in epithelial cells but also in primary macrophages. Lack of the importin- 7 gene did not show any significant differences in the cellular immune response. However, higher levels of pro-inflammatory cytokines and chemokines (MCP-1, IL12, IL6 and IL10) were detected in WT compared to $7^{-/-}$ mice potentially contributing to severe lung pathology observed in WT mice.

In summary, our findings show that the importin- 7 gene plays a crucial role in virus cell tropism and in pro-inflammatory cytokine response in the mammalian airway.

Keywords: pathogenesis, host factors, influenza virus.

O-61

Altered P-body formation in HCV-infected human liver

Gemma Pérez Vilaró*[2], Carlos Fernández Carrillo[2], Sofía Pérez del-Pulgar[2], Xavier Forns[2], Juana Díez[1]

(1) Department of Experimental and Health Sciences, Molecular Virology. Universitat Pompeu Fabra. Barcelona (2) Liver Unit. Hospital Clínic, IDIBAPS . Barcelona.

Processing bodies (P-bodies) are discrete and highly dynamic granules present in the cytosol of eukaryotic cells under normal growth conditions. They are proposed to play a key role in the control of post-transcripitional gene expression and contain translationally repressed mRNAs together with proteins from the mRNA decay and miRNA machineries. A growing body of evidence indicates that viruses establish a complex interplay with these granules. We have previously shown in cell culture that hepatitis C virus (HCV) utilizes some P-body components to propagate. Interestingly, while HCV replication is independent of P-body formation, the virus induces first changes in P-body composition and later, at advance time of infection, their disruption. These virus-induced changes of P-body abundance may have consequences *in vivo* beyond viral propagation since some studies suggested a correlation between P-body disruption and inflammatory processes. As a first step to address this question, we analyzed how HCV infection affects P-body formation in liver of infected patients. For this, we performed immunofluorescence staining with antibodies against DDX6 and Dcp1, two core P-body components, in formalin-fixed paraffin-embedded samples from liver of chronically-infected HCV patients. The abundance of P-bodies containing DDX6 or Dcp1 was reduced by 2- and 10-fold in hepatocytes of infected patients relative to hepatocytes of non-HCV controls, respectively. Importantly, such alterations in P-body numbers were hepatocyte-specific, since in immune system cells and fibroblasts present in the same liver samples the number of DDX6- and Dcp1-containg P-bodies was not affected. Thus, an alteration in P-body formation was observed only in the cell type that supports HCV infection. Interestingly, in contrast

to hepatoma cell lines, DDX6- and Dcp1-containing P-bodies do not co-localize in hepatocytes from either infected or non-HCV controls. However, they do co-localize in immune system cells and fibroblasts, uncovering a cell-dependent specialization of P-body composition. Our results demonstrate that, as found in HCV-infected hepatoma cell lines, P-body formation is impaired in human HCV-infected livers and also point out the need of *in vivo* studies to characterize P-body biology. To our knowledge this is the first report assessing *in vivo* the effect of viral infection on P-bodies.

Keywords: HCV infection, P-bodies *in vivo*, DDX6, Dcp1.

O-62

Intestinal microbiota promote baculovirus infectivity

Agata K Jakubowska*[1], Heiko Vogel[2], Salvador Herrero[1]

(1) Department of Genetics. University of Valencia. (2) Department of Entomology. Max Planck Institute for Chemical Ecology. Jena, Germany.

Baculoviruses are large DNA viruses that infect invertebrates, mainly insects from the order Lepidoptera. They were first discovered to cause insects' epizootics and are now used worldwide as biocontrol agents. Extensive studies on baculovirus biology led to the discovery that they can serve as expression vectors in insect cells; recently they have also been considered as vectors for gene therapy.

In this study *Spodoptera exigua* microarray was used to determine genes differentially expressed in *S. exigua* cells challenged with the native baculovirus *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) as well as with a generalist baculovirus, *Autographa californica* (AcMNPV). Microarray results revealed that, in contrast to the host transcriptional shut-off that is expected during baculovirus infection, *S. exigua* cells showed a balanced number of up- and down-regulated genes during the first 36 hours following the infection. Many immune-related genes, including pattern recognition proteins, genes involved in signalling and immune pathways as well as immune effectors and genes coding for proteins involved in the melanization cascade were found to be down-regulated after baculovirus infection. The down-regulation of immune-related genes was confirmed in the larval gut.

Baculovirus infection, like many other oral infections, starts with the invasion of the gut by viruses. The gut is colonized by a community of resident microbiota. We hypothesized that change in the expression of immune related genes after baculovirus infection may impact gut microbiota. Indeed gut microbial loads were found to increase after baculovirus infection. A series of bioassays showed that baculovirus increases its pathogenicity, productivity and dispersion in the presence of microbiota in the gut.

Our study reveals that baculovirus infection leads to increase of microbiota loads in the gut and that the gut microbiota play a significant role in insect

immunity and susceptibility to viral infections. These findings suggest that gut microbiota can be manipulated to improve biocontrol strategies that employ baculoviruses.

Keywords: baculovirus, nucelopolyhedrovirus, immune response, microbiota.

SESIÓN PARALELA VIII

Emerging and veterinary viruses

CHAIRS:
Ana Doménech
Juan Carlos Sáiz

O-63

Marisma mosquito virus: characterization of a novel Flavivirus isolated from *Ochlerotatus caspius* mosquitoes in Spain

Ana Vázquez*[1], Gustavo Palacios[2], Amelia P. Travassos da Rosa[3], Hilda Guzmán[3], Laura Herrero[1], Laureano Cuevas[4], Esperanza Pérez Pastrana[4], Santiago Ruíz[5], Antonio Tenorio[1], Robert B. Tesh[6], María Paz Sánchez Seco[1]

(1) Laboratorio de Arbovirus y Enfermedades Víricas Importadas. CNM, ISCIII. Madrid (2) Center for Genomic Sciences. Genomics Divisions. United States Army Medical Research Institute of Infectious Diseases (USAMRIID). Frederick, MD, USA. (3) Center for Tropical Diseases. University of Texas Medical Branch. Galveston, USA. (4) Centro Nacional de Microbiología. Instituto de Salud Carlos III. Madrid (5) Servicio de Control de Mosquitos. Diputación Provincial de Huelva. (6) Department of Pathology, Center for Biodefense and Emerging Infectious Diseases. University of Texas Medical Branch. Galveston, USA.

Flaviviruses are members of the genus *Flavivirus* (family *Flaviviridae*), many of which have been recognized as important human pathogens causing haemorrhagic fever and encephalitis. Generally, flaviviruses comprise two groups of arthropod-borne viruses (mosquito and tick-borne viruses) and a group of vertebrate viruses with no known vector (Gould et al., 2003). A small group of flaviviruses, specific for mosquitoes, is recognized as a fourth flavivirus group designated "insect specific flavivirus", which seem not to be able to infect vertebrate cells and therefore they would not represent a health risk for humans or animals. (Cook et al, 2009).

Marisma Mosquito virus (MMV) is a novel flavivirus detected and isolated from *Ochlerotatus caspius* mosquitoes from Spain. The phylogenetic analysis in a partial fragment of the NS5 gene, showed that this virus belongs to the mosquito borne flaviviruses (MBV) group (Vázquez et al.). All the MBV members are transmitted by mosquitoes and can infect vertebrates, and many of them have been recognized as important human pathogens. In this study, we describe the host range studies, molecular and serological characterization of MMV.

The virus was isolated in several mosquito cell lines, but growth in selected vertebrate cell lines was almost inappreciable. Moreover, the isolation by intracerebral infection of newborn mice was unsuccessful. The phylogenetic analysis of the whole polyprotein sequence showed that MMV is grouped with others new flaviviruses detected recently, like Lammi virus (2004) from Finland and Chaoyang virus (2008) and Donggang virus (2009) from China. They represent a distinct phylogenetic lineage related to previously identified members of the MBV. Serological studies (IFA, HI, CF and neutralization assays) showed that MMV is highly related antigenically to the Japanese encephalitis serocomplex viruses, mainly with West Nile (WNV) and Usutu viruses (USUV).Unfortunately, other viruses in the phylogenetic group were not available for antigenic studies.

Future studies will be focused to determinate the rate of infection of MMV in humans or animals and to check if the virus is able to infect bird and

mammal animal models. Moreover, we are very interested to analyze if mosquito cell lines or mosquitoes naturally infected with MMV could be refractory or more susceptible to superinfection with another pathogenic flaviviruses, like WNV or USUV also circulating in Spain, due to viral interference.

Keywords: flavivirus, Spain, mosquito, marisma mosquito virus, West Nile virus.

0-64

Experimental infection of red-legged partridges with Euro-Mediterranean isolates of West Nile virus belonging to different lineages (1 and 2)

Francisco Llorente*[1], Elisa Pérez Ramírez[1], Javier del Amo[1], Jordi Figuerola[2], Ramón Soriguer[2], Miguel Ángel Jiménez Clavero[1]

(1) Centro de Investigación en Sanidad Animal (CISA). I.N.I.A. Valdeolmos, Madrid (2) Estación Biológica de Doñana. C.S.I.C. Sevilla.

West Nile virus (WNV) is an arthropod-borne virus of the genus Flavivirus. Its natural cycle involves several species of birds as hosts and mosquitoes as vectors, with horses and humans as dead-end hosts. In recent years, in Europe an increase in the number of outbreaks has been observed. In the same area, WNV disease in wild birds has been observed with increasing frequency, involving not only lineage 1 strains, but also strains belonging to the newly introduced lineage 2.

The red-legged partridge (*Alectoris rufa*) is an autochthonous Mediterranean species that plays a key role in Mediterranean ecosystems and is an important small game bird in Southern Europe. The red-legged partridge has been shown to be susceptible to Spanish and North African WNV strains [1] highly related phylogenetically [2] belonging to lineage 1. The present study describes the course of an experimental infection of red-legged partridges with other Euro-Mediterranean WNV strains belonging to lineages 1 and 2.

Groups of nine seven-week old partridges each were inoculated subcutaneously with three different WNV isolates: Italy/2008 (lineage 1), Israel/1998 (lineage 1) and Austria/2008 (lineage 2). A control group was sham-inoculated and handled in the same way as the virus-inoculated groups. Clinical outcome was evaluated and weight monitored in all the birds. Duration and intensity of viremia, virus shedding and viral distribution in organs were analysed by real time RT-PCR. Humoral immune response at the end of the experiment was also assessed.

Animal inoculated with the three strains became viremic and showed clinical symptoms and variable mortality. Viral load in organs indicates a systemic infection in dead partridges. Cloacal and oral shedding was observed after the inoculation with the three strains, but the presence of viral genome was more consistently detected in oral than in cloacal swabs. These results show that: 1) Euro-Mediterranean strains from lineages 1 and 2 are pathogenic for the red-legged partridge and 2) this species can be a competent reservoir for WNV in Europe.

1- Sotelo et al. (2011) Pathogenicity of two recent Western Mediterranean West Nile virus isolates in a wild bird species indigenous to Southern Europe: the red-legged partridge. *Vet Res* 42:11
2- Sotelo et al. (2011) Phylogenetic relationships of Western Mediterranean West Nile virus strains (1996-2010) using full-length genome sequences: single or multiple introductions?. *J Gen Virol* 92:2512-22

Keywords: West Nile virus, partridge.

O-65

Differentiation of infected and vaccinated animals (DIVA) with commercially attenuated vaccines using the nsp7 protein of PRRSV

Marga García Durán*[1], Nuria de la Roja[1], Javier Sarraseca[1], Emanuela Pireli[2,3], Ivan Díaz Luque[2], Iván Hernández[4], María José Rodríguez[1]

(1) Departamento de Investigación. INGENASA. Madrid (2) ENDEMOVIR. Centre de Recerca en Sanitat Animal (CReSA), UAB-IRTA. (3) Department de Sanitat i Anatomia Animals. Universitat Autònoma de Barcelona. (4) Servicio Técnico Porcino. Veterinaria. Boehringer Ingelheim España, S.A. Barcelona.

PRRSV is the major cause of reproductive and respiratory disorders in pigs worldwide causing important economical losses. Both inactivated and attenuated vaccines (MLV) have been designed for the two PRRS genotypes described (I and II) in order to control the disease. The development of an assay able to differentiate between infected and vaccinated animals is important so that vaccines can be used in control programmes. Non structural protein 7 (nsp7) from PRRSV is an attractive candidate for a DIVA test because it is not packaged in the virion, is relatively conserved within the two genotypes and the specific antibody response generated is measurable from 10-14 days post infection *(Clin Vaccine Immunol. 2009May;16(5):628-35)*. Although the assay was initially designed for inactivated vaccines (theoretically containing no or low levels of nsp7), unexpected differences between the nsp7 antibody levels generated in infected and MLV vaccinated pigs were found. Thus, the response to N and nsp7 (PRRS type I or II) was compared in experimental and field sera collections (*col*) at different days post immunization: *col1)* SPF pigs vaccinated with *A*-Porcilis (type I, 5 pigs) or *B*-Ingelvac (type II, 5 pigs) under experimental conditions; *col2)* 15 farm sows vaccinated with one dose of Ingelvac; *col3)* Field sera from PRRS type II infected pigs.In experimental or farm controlled vaccination conditions (*col1* and *col2*) pigs were positive by N assay (with the only exception of the earliest time point post vaccination with Porcilis) and negative by nsp7, supporting nsp7 based assay as a DIVA. The comparison of sera from infected and vaccinated animals (*col1B+col2* versus *col3*) showed that some infected pigs remained negative to nsp7. These could correspond to animals in early stages of infection, when they had measurable response to N protein but no to nsp7 protein. When the mean values of vaccinated and infected

groups were compared, the differences were significant.Up to now, we have demonstrated that the assay based on nsp7 protein works as a DIVA at the individual level for MLV vaccines in experimental conditions and it seems to work at the population level with farm sera. Future work will be done to fully determine the possibilities of this test by including a higher number of characterized sera.

Acknowledgements The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/ 2007-2013) under grant agreement nº 245141

Keywords: PRRSV, DIVA, nsp7.

O-66

In vivo attenuation of Rift Valley fever virus (RVFV) through administration of foot and mouth disease virus (FMDV) non-coding synthetic RNAs in mice

Gema Lorenzo[1], Miguel Rodríguez Pulido[2], Elena López[1], Francisco Mateos[1], Francisco Sobrino[2], Belén Borrego[1], Margarita Saíz[2], Alejandro Brun*[1]

(1) Centro de Investigación en Sanidad Anima (CISA). INIA. Valdeolmos, Madrid (2) CBM. CSIC. Cantoblanco, Madrid.

In this work we have addressed the effect of synthetic, non-infectious RNA transcripts, mimicking structural domains of the non-coding regions (NCRs) of the foot-and-mouth disease virus (FMDV) genome, on the antiviral, as well as innate and adaptive immune responses against infection by Rift Valley fever virus (RVFV). Inoculation of mice with these RNAs have been previously shown to significantly reduce their susceptibility to foot-and-mouth disease virus and West Nile virus infections, respectively. Groups of 5 mice were inoculated intraperitoneally with 200ug of synthetic RNA resembling the 5´-terminal S fragment, the internal ribosome entry site (IRES) and the 3' NCR of the FMDV genome. The RNA inoculation was performed 24 hours before (-24h), 24 hours after (+24h) or simultaneous (0) to the challenge with a lethal dose of the virulent RVFV isolate 56/74. Administration of the IRES RNA provided higher survival rates than administration of S or 3' NCR transcripts, either at -24h or +24h after challenge. In contrast, when RNA inoculation and viral challenge were performed simultaneously, all mice survived in both IRES- and 3' NCR-inoculated groups, with an 80% survival in the S-fragment group. Surviving animals from each group were then re-challenged with a lethal dose of RVFV. All of the mice that survived the challenge showed neutralizing antibody responses. The use of these FMDV NCRs RNAs as potential vaccine adjuvants will be discussed.

Keywords: Rift Valley fever, FMDV non coding RNAs.

0-67

Modified vaccinia Ankara expressing African horse sickness virus (AHSV) VP2 (MVA-VP2) induces a highly protective humoral immune response against AHSV in a mouse model upon passive immunisation

Eva Calvo Pinilla*[1], Francisco de la Poza[2], Peter Mertens[1], Javier Ortego[2], Javier Castillo Olivares[1]

(1) Department of Arbovirology. The Pirbright Institute. Pirtbright. UK (2) Departamento de Inmunoprofilaxis de enfermedades virales transmitidas por vectores. Instituto de Investigacion en Sanidad Animal (CISA) INIA. Valdeolmos, Madrid.

African horse sickness virus (AHSV) is an arthropodborne pathogen that affects all species of equidae and causes high mortality in horses. Recently, a recombinant vaccine based on a highly attenuated modified vaccinia Ankara virus expressing the protein VP2 of AHSV-4 (MVA-VP2) elicited neutralising antibody responses in horses and demonstrated its efficacy protecting interferon alpha receptor gene knock-out mice (IFNAR -/-) against challenge [1,2]. Vaccinated IFNAR -/- mice did not show infectious virus in blood or any clinical signs of disease after infection with ASHV-4.

In this study, we have evaluated the protective role against AHSV of the humoral immunity induced by MVA-VP2 vaccination in IFNAR -/- mice. Thus, the antibody and cell-mediated immune responses induced in MVA-VP2 vaccinated mice were analysed, and their protective efficacy evaluated against virulent challenge. In addition, a passive immunisation experiment was conducted whereby donor antiserum from MVA-VP2 vaccinated or MVA-VP2 vaccinated and AHSV infected mice was transferred to AHSV non-immune recipient mice. The recipients were challenged with virulent AHSV and their protective immunity compared with MVA-VP2 vaccinated and control unvaccinated animals.

This study presents evidence that humoral immunity, possibly mediated by virus neutralising antibodies, is a pivotal factor in protection against AHSV since high level of protection is achieved after passive immunisation, although an additional cellular response is likely to be important to provide complete protection against disease.

1. Chiam R, Sharp E, Maan S, Rao S, Mertens P, et al. (2009) Induction of antibody responses to African horse sickness virus (AHSV) in ponies after vaccination with recombinant modified vaccinia Ankara (MVA). PLoS One 4: e5997
2. Castillo-Olivares J, Calvo-Pinilla E, Casanova I, Bachanek-Bankowska K, Chiam R, et al. (2011) A modified vaccinia Ankara virus (MVA) vaccine expressing African horse sickness virus (AHSV) VP2 protects against AHSV challenge in an IFNAR -/- mouse model. PLoS One 6: e16503

Keywords: AHSV, MVA, passive immunisation.

O-68

Persistence of infectious pancreatic necrosis virus: the effects of an oral DNA vaccine on fish survivors

Natalia Andrea Ballesteros Benavides*[1], Sara Isabel Pérez Prieto[1], Sylvia Patricia Rodríguez Saint Jean[1]

[1] Departamento de Microbiología molecular. Centro de Investigaciones Biológicas CSIC. Madrid.

Infectious pancreatic necrosis virus (IPNV) belongs to the *Aquabirnavirus* genus and it is the archetypal species of the *Birnaviridae* family. The members of this family are naked, icosahedral viruses with a two-segmented, double-stranded RNA genome. IPNV is found worldwide, and the damage it can cause is enhanced by its capacity to evade the host's immune defences and establish persistence. This persistence or latent viral infection is particularly relevant in aquaculture, as carrier fish may periodically shed infectious virus through their faeces and reproductive tracts, thereby transmitting the virus to their progeny and other susceptible fish. Ideally, a vaccine against IPNV would not only be able to decrease mortality but also, it should prevent the development of carrier fish. Oral DNA vaccines are now beginning to be tested in fish, and in this sense, we have been assessing the activity of a plasmid expressing the IPNV-VP2 gene encapsulated in alginate microspheres (de Las Heras et al., 2009; Ballesteros et al., 2012). To better understand the carrier state and the action of the vaccine, we have examined the expression of a group of immune-related genes identified in these earlier studies, as well as quantifying the amount of IPNV in asymptomatic rainbow trout survivors, both in control infected fish and fish challenged with the virus after oral vaccination. There were significant differences in the relative expression of certain genes between the control fish and those that were infected after vaccination, with the vaccine mimicking the virus's action. Fewer IPNV transcripts were detected 45 days post-infection in vaccinated challenged rainbow trout than in the surviving control infected fish. Infective virus was recovered from infected survivor fish but not from the vaccinated fish, which suggests an active role of the vaccine in controlling IPNV infection.

This work was supported by grant AGL2010-18454 from the MINECO (Spain).

De las Heras, A, Rodriguez Saint-Jean, S and Pérez-Prieto, SI. (2010). Fish & Shellfish Immunol 28:562-570

Ballesteros NA, Rodriguez Saint-Jean, S., P, Perez-Prieto, SI & Coll JM. Fish & Shellfish Immunol (2012), 33: 1249-1257

Keywords: persistence, IPNV, DNA vaccine.

O-69

Relevance of severe acute respiratory syndrome envelope protein domains in virus virulence

José A. Regla Nava*[1], Marta L. de Diego[1], José L. Nieto Torres[1], José M. Jiménez Guardeño[1], Raúl Fernández Delgado[1], Luis Enjuanes[1]

(1) Department of Molecular and Cell Biology. Centro Nacional de Biotecnología (CNB-CSIC), Universidad Autónoma de Madrid

A severe acute respiratory syndrome coronavirus (SARS-CoV) lacking the structural envelope E gene (rSARS-CoV-MA15-deltaE) is attenuated in three animal models. In addition, it was shown that SARS-CoV envelope (E) protein is a virulence factor affecting cellular stress, unfolded protein responses and proinflammatory responses. To identify the domains of SARS-CoV E protein responsible for attenuation. Mutant viruses (rSARS-CoV-MA15-E*) with amino acid substitutions in the amino terminal (rSARS-CoV-MA15-MutA), and with small deletions covering the internal region of E protein were constructed using an infection cDNA encoding a mouse adapted virus strain (MA15). Infection of conventional mice with the MA15 strain reproduces many aspects of the human disease such as high titers and pathological changes in lung, viremia, neutrophilia and lethality. Viruses with a mutated E protein amino terminus (rSARS-CoV-MA15-MutA), or with and deletions in the central domain of the carboxy terminal region (rSARS-CoV-MA15-deltaC, deltaD, deltaF) grew with titers similar to rSARS-CoV-wt *in vitro*. In contrast, viruses lacking the first and the last domain of the E protein carboxy terminal region (rSARS-CoV-MA15-deltaB and deltaG) grew with titers 100-fold lower, similar to the ones observed for the rSARS-CoV-MA15-deltaE. These data suggested that E protein domains B, C, D and F were necessary for an efficient virus growth. The virulence of rSARS-CoV-MA15-E* mutants was analyzed in Balb/c mice. Infection with mutants rSARS-CoV-MA15-deltaB, delta C, deltaD and deltaF did not cause weight loose and all of the mice survived indicating that deletion of E protein domains B, C, D and F led to attenuated viruses. In contrast, deletion of the last segment of the E protein carboxy terminal domain G did not attenuated the virus as infected mice lost weight and died similarly to mice infected with the wt virus. Amino acid substitutions at the E protein amino terminal domain led to an intermediate phenotype. To evaluate the molecular mechanisms leading to SARS-CoV attenuation, viral growth, histopathology and pro-inflammatory cytokine expression in the lungs of infected BALB/c mice are being evaluated. Interestingly, the attenuated mutants, rSARS-CoV-MA15-deltaB, deltaC, deltaD and deltaF, provided full protection against the challenge with a virulent virus, indicating that they are promissing vaccine candidates.

Keywords: SARS-CoV, virulence, envelope protein.

O-70

Deciphering the molecular mechanisms involved in rabbit resistance to prions: search of the key amino acids by *in vitro* replication techniques

Hasier Eraña*[1], Natalia Fernández Borges[1], Saioa R. Elezgarai[1], Sonia Veiga[2], Ester Vázquez[2], Chafik Harrathi[1], Gabriel Ortega[3], Mayela Gayosso-Miranda[1], Óscar Millet[3], Witold Surewicz[4], Jesús R. Requena[2], Joaquín Castilla[1,5]

(1) Department of Proteomics. CIC bioGUNE. Derio. Vizcaya (2) CIMUS Biomedical Research Institute & Department of Medicine. University of Santiago de Compostela-IDIS. (3) Nuclear Magnetic Resonance. CIC bioGUNE. Derio, Vizcaya (4) Department of Physiology & Biophysics. Case Western Reserve University. Cleveland, USA (5) IKERBASQUE. Basque Foundation for Science. Bilbao.

Since the 60's, Transmissible Spongiform Encephalopaties (TSEs), caused by amyloidogenic infectious conformer (PrP^{Sc}) of the globular and benign PrP (PrP^{C}), also named prion, had been described in several mammalian species appearing either naturally (scrapie in sheep, BSE in cattle, CWD in cervids, CJD in human) or by experimental transmission studies (scrapie in mouse or hamster). Prion diseases can be transmitted from one species to another albeit not with the same efficiency in them all. In the last 40 years many aspects of the prionopathies have been solved: 1) the etiological agent, 2) prions occur in the form of different strains that show distinct biological and physicochemical properties, even when encoded by PrP with the same amino acid sequence. There are still many open questions regarding the strain phenomenon and interspecies transmissibility. Apart from transmissibility studies performed by experimental challenges, the BSE outbreak occurred in the UK in the 90's showed that while some species were readily affected (mice or felines) others did not develop TSE (rabbits, dogs, horses) even when exposed to the same agent and with PrP sequences very similar to susceptible ones.

In an attempt to study the mechanisms involved in the high resistance to TSEs of certain species, the mouse-rabbit transmission barrier is being used as a model. We are studying which are the specific amino acids that determine the susceptibility/resistance to prion propagation. For that, Protein Misfolding Amplification (PMCA) is being used, as it mimics PrP^{C} to PrP^{Sc} conversion *in vitro* with accelerated kinetics. Specifically, PMCA based on recombinant PrP (recPMCA) is being used instead of brain-derived PrP. This new technology allows easy amino acid substitutions in rabbit PrP and rapid analysis of their misfolding propensity when inoculated with a wide range of prion strains. Since the wild type (wt) rabbit recombinant PrP could not be misfolded by serial recPMCA rounds with at least a dozen of prions from a diversity of origins, a series of rabbit recombinant PrP mutants have been designed containing every single amino acid substitution compared to mouse recombinant PrP. At least a promising key substitution has been found that makes rabbit recombinant PrP highly susceptible to misfolding. Further studies are ongoing in order to decipher why a particular substitution but not others appear to alter dramatically the misfolding properties of the rabbit recombinant PrP.

Keywords: prion, transmission barrier, PMCA

SESIÓN PARALELA IX

Viral replication

CHAIRS:
Luis Enjuanes
Luis Menéndez-Arias

O-71

Effects of RNase H-inactivating mutations on the fidelity of HIV-1 group O reverse transcriptase

Mar Álvarez*[1], Verónica Barrioluengo[1], Raquel N. Afonso-Lehmann [2], Luis Menéndez-Arias[1]

(1) Departamento de Virología y Microbiología. Centro de Biología Molecular Severo Ochoa (CSIC-UAM). Madrid (2) Departamento de Parasitología, Ecología y Genética. Instituto Universitario de Enfermedades Tropicales y Salud Pública de Canarias. Universidad de la Laguna. Tenerife.

Asp^{443} andGlu^{478} are essential active site residues in the RNase H domain of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT). It is widely accepted that RNase H activity may affect mutation and recombination through its coordinated action with the RT DNA polymerase activity during minus-strand DNA synthesis and plus-strand DNA transfer. However, there is little information about its contribution to DNA-dependent DNA synthesis. We have used the forward mutation assay to study the effects of substituting Asn for Asp^{443} or Gln for Glu^{478} on the fidelity of DNA-dependent DNA synthesis of phylogenetically diverse HIV-1 RTs (*e.g.* group O and group M subtype B RTs). Mutations D443N and E478Q abolish RNase H activity while retaining wild-type DNA polymerase activity. HIV-1 group M (BH10) and group O RTs showed 2.0- to 6.6-fold increased accuracy in comparision with the corresponding wild-type enzymes. Analyses of their mutational spectra indicated that this is a consequence of their lower base substitution errors. However, RNase H-deficient HIV-1 group O RTs showed a higher tendency to introduce frameshift errors. One-nucleotide deletions and insertions represented between 30 and 68% of all errors identified in the mutational spectra these enzymes. In comparision with the wild-type HIV-1 group O RT, RNase H-deficient enzymes showed decreased processivity and higher dissociation rate constants (k_{off}) from the template-primer, thereby facilitating strand transfer and template or primer slippage. Our data suggest that mutations in the RNase H domain could also contribute to HIV variability during plus-strand DNA synthesis or during the later steps of reverse transcription.

Keywords: reverse transcriptase, HIV-1, RNase H.

O-72

Functional analysis of FMDV 3D RNA polymerase

Flavia Caridi*[1], Ignacio de la Higuera[1], Maria Teresa Sánchez Aparicio[2], Esteban Domingo[1], Francisco Sobrino[1]

(1) Departamento de Virología y Microbiología. Centro de Biologia Molecular Severo Ochoa (CSIC UAM). Madrid (2) Department of Microbiology. Mount Sinai School of Medicine. New York, USA.

Picornaviruses are positive stranded RNA viruses associated with a large number of human and an-

imal diseases. These viruses encode an RNA-dependent RNA polymerase (3D RdRP) that replicates the viral genome in the infected cells. 3D polymerases are excellent targets for the development of antiviral strategies.

Here we report the functional analysis of the N-terminus of foot-and-mouth disease virus (FMDV) 3D RNA polymerase. Molecular structures of mutant 3D polymerases showed that its N-terminus conforms a loop that locates very close to the template RNA molecule (Agudo et al., 2010) and that K20 residue directly interacts with the RNA molecule to be elongated (Ferrer-Orta et al., 2006).

With this initial body of evidence, infectious clones with a 3D that included K18A, K20A or both K18A-K20A replacements were constructed and RNA transcripts were transfected in BHK-21 cells. RNAs with single replacements produced viable virus but, relative to the wt virus, cytopathic effect was delayed and the virus titers recovered were about two or five logarithm lower respectively for K18A and K20A mutants. In addition, K20A mutant showed a small plaque phenotype. No reversions were observed in the sequence of the mutant viruses rescued. On the contrary, the double substitution K18A-K20A resulted detrimental, and did not allow virus recovery. 3D polymerases with substitutions K18A, K20A, K18A-K20A as well as K18E, K20E (that allowed recovery only of revertant viruses) were expressed in E. coli cells, purified and their enzymatic activity compared with that of wild type 3D. All mutant proteins showed defects in their enzymatic activity, RNA binding and Vpg uridylylation. These defects were more pronounced in mutants K18E, K20E and K18A-K20A than in those with a single alanine replacement (K18A or K20A). Experiments are in progress to correlate structural data with the functional plasticity observed in this region of FMDV 3D involved in interactions with the RNA template.

Agudo, R., C. Ferrer-Orta et al., (2010). PLoS Pathogens 6 (8): e1001072
Ferrer-Orta, C., A. Arias, et al., (2006). Curr Opin Struct Biol 16(1):27-34

Keywords: FMDV, polymerase.

O-73

Insights into the regulatory mechanism of a non-cannonical ssRNA virus replicase

Diego Sebastián Ferrero*[1,2], Mónica Buxaderas[2], José Francisco Rodríguez[1], Núria Verdaguer[2]

[1] Departamento de Biología Molecular y Celular. Centro Nacional de Biotecnología. Madrid [2] Departamento de Biología Estructural. Institut de Biología Molecular de Barcelona.

RNA-dependent RNA polymerases (RdRps) play central roles in both, transcription and viral genome replication. The structure of these enzymes appears to be broadly conserved, following a closed right hand architecture, with fingers, palm and thumb subdomains that encircles the active site.Sequence

analyses identified five ordered sequence motifs within the palm subdomain (designated A-E) that are conserved, not only in RdRps but also in all virus replicases. However, the RdRps of a small group of ssRNA viruses (*Permutotetraviridae* family) and some dsRNA viruses (members of *Birnaviridae* family) do not follow the canonical palm organization. In these enzymes, motif C is located upstream the motif A, resulting in a palm fold with a permuted connectivity (C-A-B-D-E). Given the shortage of these atypical polymerases, there is scarce structural and biochemical information about them.

In this work we performed an extensive structural and biochemical characterization of the RdRp of Thosea asigna virus (TaV), an insect restricted (+) strand RNA virus that belongs to the *Permutotetraviridae* family. The comparative analysis of the structures of TaV RdRp, unbound and in complex with different substrates, allowed us to identify a number of elements regulating the polymerase activity. In particular, the N-terminal arm (30 aa) that blocks the active site cavity, in the unbound structure, undergoes important structural rearrangements allowing template binding as observed in the structure of the RdRp-RNA_template complex. The regulatory role of the TaV RdRp N-terminus was further confirmed by "*in vitro*" polymerization assays of the wild type and mutant polymerases. In addition, the structural similarities observed between TaV and Birnavirus RdRps, particularly in the three dimensional organization of their non-canonical palms, strongly supports the existence of a common ancestor between these apparently unrelated viruses.

Keywords: permutotetravirus, polymerase, structure.

O-74

Implication of the phospholipid PI4P in the replication of two plus strand RNA viruses: swine vesicular disease virus and foot-and-mouth disease virus

Mónica González Magaldi*[1], Francisco Sobrino[1], Miguel Ángel Martín Acebes[1]

[1] Departamento de Virología y Microbiología. Centro de Biología Molecular Severo Ochoa. Madrid.

Cells infected with positive-strand RNA viruses undergo a dramatic remodeling of their intracellular membranes, and viral RNA replication frequently takes place at the cytosolic leaflet of these remodeled membranes. Based on results obtained with coxsackievirus (CV) B3, Poliovirus (PV) and hepatitis C virus, it has been recently proposed that a common specific lipid microenvironment enriched in phosphati-dylinositol-4-phosphate (PI4P) is crucial for the replication of RNA viruses (Arita et al., 2011;Reiss et al., 2011).

PIK93, a PI4P kinase III (PI4KB) inhibitor, has been recently identified as a potent anti-PV compound that targets PI4KB to suppress interaction of PI4P with the viral 3D polymerase on the reorganized membrane vesicle that leads to viral replication complex formation (Hsu et al., 2010).

In the present report we have analyzed the possible implication of PI4KB in replication of two positive strand RNA viruses: FMDV, prototypic member of the aphtovirus genus and SVDV a

member of the genus Enterovirus within the family Picornaviridae that is assumed to be derived from the human pathogen CVB5.

A plasmid encoding GFP-tagged FAPP1-PH that binds to PI4P lipids was used to transfect susceptible cultured cells to determine PI4P localization (Martin-Acebes et al., 2011). PI4P signal was observed at discrete structures surrounding cell nuclei that colocalized with the Golgi marker GM130, indicating a primary distribution of this lipid at the Golgi complex. Confocal laser scanning microscopy of cells transfected with the PI4P reporter plasmid and later infected with FMDV did not show a redistribution of PI4P. On the contrary, as expected, a redistribution of PI4P was found in cells infected with SVDV in which colocalization between viral protein 3A and and FAPP1 was noticed.

When the effect of the inhibition of PI4P synthesis by PIK93 was analyzed with FMDV or SVDV, no reduction of production was observed when the drug was added at 0 or 3 h p.i at concentrations (0.5 and 1 μM) that inhibited CVB5 yield in a dose-dependent manner.

Resistance of CVB3 to enviroxime is associated with cross resistance to inhibitors of PI4KB such as GW5074 and PIK93, and replacements V45A and H57Y in protein 3A have been associated to this phenotype (van der Schaar et al., 2012). Sequence of the protein 3A coding region of the SVDV isolate used in this study revealed the presence of I45 suggesting that this residue could contribute to the resistance to PIK93 observed.

Keywords: PI4P, PI4KB, SVDV, FMDV, PIK93.

O-75

Role of non-structural protein nsP1a/4 in intestinal pathogenicity induced by human astrovirus infection

Anna Pérez Bosque[1,2], Lluïsa Miró[1,2], Rosa M. Pintó[3], Susana Guix*[2,3]

(1) Department of Physiology. University of Barcelona. (2) Institut de Recerca en Nutrició i Segyretat Alimentària (INSA-UB). Barcelona. (3) Department of Microbiology. University of Barcelona.

Diarrheal diseases are characterized by active secretion of chloride and/or bicarbonate into the intestine and a subsequen loss of fluid. Human astroviruses (HAstV) are constitute a common cause of acute gastroenteritis worldwide and are typically associated with children, as well as elderly and immunocompromised individuals. In spite of the well-recognized impact of HAstV on human health, our understanding of how these viruses cause diarrhea and disease is still limited. Astroviruses are non-enveloped icosahedric small viruses with a positive-sense single-stranded RNA genome that belong to the family *Astroviridae*. Recent data support the involvement of a hypervariable region (HVR) located within one of HAstV nonstructural proteins (nsP1a/4 protein) in viral RNA replication processes. Significant differences are observed in the viral load of clinical samples corresponding to certain nsP1a/4-derived genotypes, confirming the influence of HVR variability on the viral replication phenotype.

With the aim of better understanding the molecular mechanisms of HAstV induced pathogenesis, we studied whether variability in nsP1a/4 protein affected intestinal permeability, ion absorption, and/or cellular response to infection. Three viral mutants containing different nsP1a/4 variants as well as the wildtype virus were tested on an *in vitro* model of intestinal epithelial barrier using highly differentiated CaCo-2 cells. Our results show that viral progeny is released almost exclusively from the apical surface of polarized intestinal cells. While alteration of permeability is highly dependent on the multiplicity of infection and a minimal percentage of infected cells is required for all mutants to cause significant changes in epithelial integrity, our data indicate that HAstV infection induces overexpression of the ENaC gene (Epithelial Sodium Channel). Finally, we observed that cells respond to HAstV infection by expressing interferon with differences according to nsP1a/4 variability, suggesting that cellular antiviral interferon response may play an important role in controlling viral infections *in vivo*. Results from these studies provide insights on the mechanisms by which HAstV cause diarrhea and disease, with special emphasis on nsP1a/4 protein function.

Keywords: astrovirus, pathogenicity, interferon.

O-76

High-order structures in the coronavirus genome mediated by a novel long distance RNA-RNA interaction promote discontinuous RNA synthesis during transcription

Lucía Morales*[1], Pedro A. Mateos Gómez[1], Sonia Zuñiga[1], Luis Enjuanes[1], Isabel Sola[1]

(1) Department of Molecular and Cell Biology. National Center of Biotechnology (CNB-CSIC). Madrid.

Coronavirus (CoV) transcription is a discontinuous process of RNA synthesis unique among positive RNA viruses. All the subgenomic mRNAs have a leader sequence at the 5' end. This leader is present only once at the 5' end of the genome and is added by a template-switch mechanism during the production of minus polarity sgRNAs. This process consists in a similarity-assisted RNA recombination between distant genomic regions. This high frequency recombination requires sequence complementarity between the transcription regulating sequences (TRS) at the leader region and those preceding each gene in the nascent minus RNA. Furthermore, the template switch requires the physical proximity of RNA genome domains located between 20 to 30 thousand nucleotides apart. It has been shown that the efficacy of this recombination step was promoted by novel long-distance RNA-RNA interactions between RNA motifs located close to the TRSs regulating the expression of the nucleoprotein (N) gene, and complementary sequences mapping at the 5' end of the genome. These inter-

actions would bring together the motifs involved in the recombination process. This finding indicates that the formation of RNA high-order structures in the CoV genome is necessary for the optimal expression of, at least, viral N gene. The requirement of these long distance interactions for transcription was shown by the engineering of CoV replicons in which the complementarity between the newly identified sequences was disrupted. Furthermore, disruption of complementarity in mutant viruses led to mutations that restored complementarity, wild-type transcription levels and viral titers by passage in cell culture. The relevance of these high-order structures for virus transcription was reinforced by the phylogenetic conservation of the involved RNA motifs in CoVs.

Keywords: coronavirus, transcription, long distance RNA-RNA interaction, high-order structures.

O-77

Simultaneous and persistent infections of picornaviruses in the lepidoptera *Spodoptera exigua*

Agata K Jakubowska[1], Melania D'Angiolo[1], Anabel Millán Leiva[1], Salvador Herrero*[1]

(1) Department de Genetics. Universitat de Valencia. Burjassot, Valencia.

Viral covert infections in invertebrates have been traditionally attributed to the result of sublethal infections that were not able to establish a patent infection. Recent studies are revealing that, although true for some viruses, other viruses may follow the strategy of establishing covert or persistent infections without producing the death of the host. During last years, with the development of the Next Generation Sequencing (NGS) technologies, the number of cases describing new viral covert infections in all type of hosts has increased drastically.

The beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae) is a worldwide pest that causes significant loses to agricultural and ornamental plant industries. In Spain, its effect is especially relevant in the horticultural crops grown in Almería. Recently, we have used NGS to obtain a comprehensive transcriptome of the larval stage, revealing the presence of an important number of unigenes belonging to novel RNA viruses, most of them from the order *Picornavirales*. In order to characterize such viral complex, we have completed the genomic sequence of three picornaviruses, two of them representing new members of the family *Iflaviridae* and a third one defining a new family. Additional studies have been performed to determine their sequence variability, infectivity, tissue distribution, structure, and the interaction between them. Influence of these viruses on the insect fitness as well as their effect on other viral and bacterial entomopathogens used for the control of this pest is also discussed.

Keywords: insect virus; iflavirus, nora virus, picornavirus, covert infection, persistent infection.

0-78

Regulation of HCV NS5B RNA-polymerase activity by protein-protein interactions

Alberto J. López Jiménez[1], Pilar Clemente Casares[1], Rosario Sabariegos[1], María Llanos Valero[1], Mathy Froeyen[2], Antonio Mas*[1]

(1) Centro Regional de Investigaciones Biomédicas. Universidad de Castilla-La Mancha. Albacete (2) Rega Institute for Medical Research. K.U. Leuven. Belgium.

The hepatitis C Virus (HCV) RNA-dependent RNA-polymerase (NS5B) is responsible for the replication of the (+) strand genome via a (-) strand intermediate through an RNA-dependent RNA-polymerase (RdRp) activity. *in vitro* RNA synthesis by NS5B may be performed by elongation of a template-primer complex or initiated de novo. However, RNA replication *in vivo* occurs by a de novo mechanism. This process implies conformational changes that direct transition from a "closed-form" initiating complex to a processive "open-form" elongation complex. This transition should involve rearrangement of some polymerase sub-units including the beta-loop and the c-terminal domain. It was previously shown that oligomerization of NS5B is relevant in the context of *de novo* initiation. Using polymerase activity assays in a sym/sub system, we have analyzed the importance of intermolecular interactions to control conformational changes, dissecting biochemical characteristics of the initiation and the elongation activities. On other hand, protein-protein docking and molecular dynamics were used in a search of energetically plausible interaction poses for this interaction. Based on this model and previous experimental data from our laboratory we have designed a series of interaction mutants to address the relevance of NS5B-NS5B interactions in the different steps of RNA synthesis. These mutations were also introduced in a subgenomic replication system to analyze the effect of these changes in an *in vivo* system. According with the obtained results we propose a coherent model where different structural elements from one NS5B monomer, mainly the -loop and the T-helix in the thumb subdomain, would be connected to elements from the other NS5B monomer, mainly the F-helix in the fingers subdomain. This NS5B-NS5B interaction via a fingers-thumb connection regulates early steps of RNA synthesis, controlling the rate limiting process of transition from the distributive initiation to the processive elongation. Furthermore, these results allow us to define protein surfaces that could be potential targets for the design of a new class of inhibitors of the viral replication.

Keywords: HCV NS5B replication.

SESIÓN PARALELA X

Biophysics of viruses and nanovirology

CHAIRS:
Mauricio García-Mateu
David Reguera

O-79

Structure of *Penicillium chrysogenum* virus by cryo-electron microscopy

José Ruiz Castón*[1], Josué Gómez Blanco[1], Daniel Luque[1], Damiá Garriga[2], José M. Gonzaléz[1], Axel Brilot[3], Wendy M. Havens[4], José L. Carrascosa[5], Benes L. Trus[6], Nuria Verdaguer[2], Nikolaus Grigorieff[3], Said A. Ghabrial[4]

(1) Department of Structure of Macromolecules. Centro Nacional de Biotecnología/CSIC. Madrid (2) Department of Structural Biology. IBMB/CSIC. Barcelona (3) Rosenstiel Center. Brandeis University. Waltham, USA (4) Department of Plant Pathology. University of Kentucky. Lexington. USA (5) Department of Structure of Macromolecules. Centro Nacional de Biotecnología/CSIC. Madrid (6) Center for Information Technology. National Institutes of Health. Bethesda. USA.

Chrysoviruses are fungal double-stranded RNA viruses with a multipartite genome comprised of four monocistronic dsRNA segments. Each segment is separately encapsidated in a similar particle. Penicillium chrysogenum virus (PcV) is the type species of the family *Chrysoviridae*. The PcV capsid is based on a T=1 lattice formed by 60 subunits, and the capsid protein appears to be a repeated a-helical domain, indicative of gene duplication. Whereas the PcV capsid protein has two motifs with a similar fold, most dsRNA virus capsid subunits consist of dimers of a single protein (a 120-subunit capsid). This ubiquitous stoichiometry provides an optimal framework for genome replication and organization. In addition, the capsid remains undisturbed throughout the viral cycle and isolates the genome from cell defense mechanisms.

We report the three-dimensional structures by single-particle cryo-electron microscopy (cryo-EM) analysis of PcV at ~4 Å resolution. Our atomic model of the PcV virion is suitable for a nearly complete trace of the 982-amino-acid capsid protein. The full-atom model of the capsid showed the critical contacts among structural subunits that mediate capsid assembly, and specific RNA-protein interactions on the inner surface. Despite the lack of sequence similarity between the two halves, the capsid protein is an almost perfect structural duplication of a single domain in which most a-helices and b-chains matched very well. Superimposition of secondary structure elements showed, in addition to the N- and C-terminal arms, a single "hot spot" into which structural and functional variations can be introduced by insertion of distinct segments. Overlaying LA capsid protein (a totivirus with an undivided genome) on either of the PcV elements, while maintaining the same spatial arrangement in the shell, highlighted the same conserved PcV motif and hot spot for insertions, in addition to two new insertion zones. Structural comparison of PcV and other dsRNA viruses suggested that the PcV conserved core is partially preserved in *Reoviridae*. Our analysis detects three preferential sites at which the complexity of the preserved ahelical core might have increased and evolved to the highly varied structures observed today.

The near-atomic structure of the PcV capsid protein derived from cryo-EM data has allowed us to determine that its conserved core is a hallmark fold preserved in the dsRNA virus lineage.

Keywords: capsid structure, dsRNA virus, evolution, cryo-EM.

O-80

Characterization and mapping of the dsRNA-binding domain of the infectious bursal disease virus VP3 polypeptide

Idoia Busnadiego[1,2], María T Martín[3], Diego S Ferrero[1], María G. Millán de la Blanca*[1], Núria Verdaguer[4], Leonor Kremer[3], José Francisco Rodríguez[1]

(1) Departamento de Biología Molecular y Celular. Centro Nacional de Biotecnología. Madrid (2) Institut of Infection, Immunity and Inflammation. University of Glasgow. Scotland (3) Protein Tools Unit. Centro Nacional de Biotecnología. Madrid (4) Departamento de Biología Estructural. Institut de Biología Molecular de Barcelona.

The infectious bursal disease virus (IBDV), the best characterized member of the *Birnaviridae* family, is responsible for an immunosuppressive syndrome that affects juvenile domestic chickens causing major losses to the poultry industry.

We have previously shown that the IBDV VP3 (28 kDa) polypeptide is a multifunctional protein that plays a central role during the virus life cycle: (i) acting as a scaffolding factor for particle assembly; (ii) activating the RNA dependent RNA polymerase (RdRP); and (iii) interacting with the dsRNA genome segments for the assembly of the transcriptionally-active ribonucleoprotein (RNP) complexes that occupy the inner capsid space.

Recently, we have found that the VP3 protein is also a key player in the mechanism(s) used by this virus to evade the innate antiviral host's response, acting both as an efficient silencing suppressor (Valli et al., PLoS One. 2012. 7:e45957) and preventing the activation of the protein kinase RNA-activated (PKR) in response to different stimuli (PLoS One. 2012. 7:e46768). Interestingly, these two activities are strictly dependent upon the ability of this polypeptide to interact with molecules of dsRNA. This observation prompted us to perform an in depth study to ascertain the most relevant hallmarks of the VP3/dsRNA interaction as well as to precisely map the VP3 dsRNA-binding domain. For this, we have used two complementary experimental approaches, surface plasmon resonance and electrophoretic mobility shift assays (EMSA), using synthetic dsRNA of different lengths and sequence composition. Our study also involved the use of series of recombinant versions of the IBDV polypeptide. The results of this analysis demonstrate that the VP3 polypeptide binds RNA duplex molecules with a minimal size of 9 base pairs in a sequence-independent mode. We have also located two discrete electropositive regions within surface of the VP3 dimer that are directly involved in dsRNA binding. The use of mutant VP3 polypeptides allowed the identification of two well exposed basic residues that are absolutely essential for the establishment of the VP3/dsRNA interaction. Using the described experimental data along with the reported crystal structure of the VP3 dimer, a candidate VP3/dsRNA complex model has been built. This might help in further assessing the role of VP3 in RNP packaging and RdRP activation as well as its possible participation in the translation mechanism of IBDV mRNAs.

Keywords: dsRNA-protein interactions, multifunctional protein, dsRNA bindig domain.

O-81

First structural characterization of adenoviruses infecting lower vertebrates

Rosa Menéndez-Conejero[1], Judit Pénzes[2], Inna Romanova[3], Tibor Papp[2], Andor Doszpoly[2], Alberto Paradela[1], Rachel Marschang[3], Balázs Harrach[2], María Benkö[2], Carmen San Martín*[1]

[1] Department of Macromolecular Structures. Centro Nacional de Biotecnología (CNB-CSIC). Madrid [2] Institute for Veterinary Medical Research. Hungarian Academy of Sciences. Budapest. Hungary [3] Institute for Environmental and Animal Hygiene. University of Hohenheim. Stuttgart. Germany.

Adenoviruses (AdV) are widely used as vehicles for gene therapy or vaccination. However, their successful use in humans is impaired by pre-existing immunity to the most common vectors. An approach to solve this problem is the use of recombinant AdV of non-human origin (1). AdVs infect a large number of vertebrates, from fish to humans (2). While the structure of human AdV has been extensively characterized by electron microscopy and crystallography studies (3, 4), little is known about the structure of other AdVs. Increasing the scarce characterization of AdVs infecting hosts remote from humans (reptile, amphibian or fish) is required to estimate their possible use as vectors.

Starting from isolates from lizard and boa, we have optimized the production and purification for molecular and structural studies of two AdVs infecting reptiles (LAdV and SnAdV-1), belonging to the Atadenovirus genus. We have determined the protein composition of the virions and characterized their higher capsid stability when compared with human AdV. In LAdV, two fiber genes produce two fiber proteins that are incorporated into the virion, but surprisingly, they assemble as three fibers per vertex complex, an arrangement that had never been observed in adenovirus or any other eukaryotic virus. Using cryo-electron microscopy, we have determined the structure of SnAdV-1 at 12.5 Å resolution. The 3D map of SnAdV-1 reveals a set of minor coat proteins different from those in human AdV, that could account for the increased stability. Interestingly, our study indicated that one of the minor coat proteins presents a fold typical of bacteriophage host attachment proteins. This observation, together with the novel penton structure of LAdV, suggests that Atadenoviruses use a host attachment strategy largely different from that of human AdV.

1. Loser P, et al. (2002) Advances in the development of non-human viral DNA-vectors for gene delivery. Curr Gene Ther 2(2):161-171
2. Harrach B, et al. (2011) Family Adenoviridae. Virus Taxonomy: Classification and Nomenclature of Viruses. Ninth Report of the International Committee on Taxonomy of Viruses., eds King A, Adams M, Carstens E, & Lefkowitz E (Elsevier, San Diego), pp 95-111
3. Liu H, et al. (2010) Atomic structure of human adenovirus by cryo-EM reveals interactions among protein networks. Science 329(5995):1038-1043.
4. Reddy VS, et al. (2010) Crystal structure of human adenovirus at 3.5 A resolution. Science 329 (5995):1071-1075

Keywords: virus structure; adenovirus; cryo-electron microscopy.

0-82

Monitoring dynamics of human adenovirus disassembly induced by mechanical fatigue

Álvaro Ortega Esteban*[1], Ana Joaquina Pérez Berná[2], Rosa Menéndez-Conejero[2], S. Jane Flint[4], Carmen San Martin[2], Pedro José de Pablo[1]

(1) Departamento de Física de la Materia Condensada. Universidad Autónoma de Madrid. (2) Departamento de Estructura de Macromoléculas. Centro Nacional de Biotecnologia (CNB - CSIC). Madrid (4) Department Molecular Biology. Princeton University. USA.

The standard pathway for virus infection of eukaryotic cells requires disassembly of the viral shell to facilitate release of the viral genome into the host cell. Here we use mechanical fatigue, well below rupture strength, to induce stepwise disruption of individual human adenovirus particles under physiological conditions, and simultaneously monitor disassembly in real time. Our data show the sequence of dismantling events in individual mature (infectious) and immature (noninfectious) virions, starting with consecutive release of vertex structures followed by capsid cracking and core exposure. Further, our experiments demonstrate that vertex resilience depends inextricably on maturation, and establish the relevance of penton vacancies as seeding loci for virus shell disruption. The mechanical fatigue disruption route recapitulates the adenovirus disassembly pathway *in vivo*, as well as the stability differences between mature and immature virions.

Keywords: virus mechanics, virus uncoating, virus maturation, virus disruption, atomic force Microscopy, physical virology.

0-83

Mechanical disassembly of single virus particles reveals kinetic intermediates predicted by theory

Pablo J. Pérez Carrillo*[1], Milagros Castellanos Molina[1], Rebeca Pérez Fernández[1], Pedro J. de Pablo Gómez[2], Mauricio García Mateu[1]

(1) Departamento de Biología Molecular. Centro de Biología Molecular Severo Ochoa CSIC-UAM. Madrid (2) Departamento de Física de la Materia Condensada C-III. Universidad Autónoma de Madrid.

New experimental approaches are required to detect the conformational dynamics of viruses (Castellanos *et al* (2012) *PNAS* 109, 12028-33; Mateu (2012) *Virus Res.* 168, 1-22) and elusive transient intermediates predicted by simulations of virus assembly or disassembly. We have used an atomic force microscope (AFM) to mechanically induce partial disassembly of single icosahedral T = 1 capsids and virions of the minute virus of mice (MVM) (Castellanos *et al* (2012) *Biophys J.* 102, 2615-24). The kinetic intermediates formed were imaged by AFM. The results revealed that induced disassembly of sin-

gle MVM particles is frequently initiated by loss of one of the 20 equivalent capsomers (trimers of capsid protein subunits) leading to a stable, nearly complete particle that does not readily lose further capsomers. With lower frequency, a fairly stable, three-fourths-complete capsid lacking one pentamer of capsomers and a free, stable pentamer were obtained. The intermediates most frequently identified (capsids missing one capsomer, capsids missing one pentamer of capsomers, and free pentamers of capsomers) had been predicted in theoretical studies of reversible capsid assembly based on thermodynamic-kinetic models (Singh & Zlotnick(2003) *J. Biol. Chem.* 278, 18249–55), molecular dynamics (Rapaport(2008) *Phys. Rev. Lett.* 101, 186101-4*)*, or oligomerization energies (Reddy and Johnson(2005) *Adv. Virus Res.* 64, 45–68*;* Reddy *et al* (1998) *Biophys J.* 74, 546–558). We conclude that mechanical manipulation and imaging of simple virus particles by AFM can be used to experimentally identify transient, kinetic intermediates predicted by simulations of assembly or disassembly.

Keywords: sigle virus particle, structure, assembly, dissasembly, atomic force microscopy.

0-84

Physical ingredients controlling the polymorphism and stability of viral capsids

María Aznar*[1], David Reguera[1]

[1] Department de Fisica Fonamental. Universitat de Barcelona.

One of the crucial steps in the viral life cycle is precisely the self-assembly of its protein shell. Typically, each native virus self-assembles into a unique T- number structure, with some exceptions like Hepatitis B Virus, which makes T=3 and T=4 capsids. But many viruses have the capability to self-assemble into different T-number and shape structures *in vitro* by changing the assembly conditions (i.e. typically the pH, salt and protein concentrations). For example, Polyoma [1] or Simian Virus 40 [2] self-assemble *in vitro* into T=1, snub cubes, T=7 and different size tubes.

A proper understanding of the ingredients that control the *in vitro* assembly of viruses is essential to get capsids with well-defined size and structure that could be used for promising applications in medicine or bionanotechnology. However, the mechanisms that determine which of the possible capsid shapes and structures is selected by a virus and that avoid its polymorphism are still not well known.

We present a coarse-grained model to analyze and understand the physical mechanisms controlling the size and structure selection in viral self-assembly [3]. We have characterized the phase diagram and the stability of T = 1, 3, 4, 7 and snub cube structures

using Monte Carlo simulations. In addition, we have studied the tolerance of the different shells to changes in physical parameters related to ambient conditions. Finally, we will discuss the factors that select the shape of the capsid as spherical, faceted, elongated and decapsidated, in the range of parameters (directly related to measurable biophysical parameters: bending constant and spontaneous curvature) where a structure is stable.

[1] Howatson A.F. and Almeida J.D. 1960. *Observations on the fine structure of polyoma virus*. Journal of Biophysical and Biochemical Cytology, 8, 828-834
[2] Kanesashi SN.et al 2003. *Simian virus 40 VP1 capsid protein forms polymorphic assemblies in vitro*. Journal of General Virology, 84, 1899–1905
[3] M.Aznar and D. Reguera. *Physical ingredients controlling the polymorphism and stability of viral capsid*. In preparation

Keywords: polymorphism, simulations, *in vitro* self-assembly.

O-85

Hydration and wetting of a plant virus

Alexander M Bittner[1]

(1) Self-Assembly. CIC nanoGUNE . Donostia.

The Tobacco mosaic virus (TMV) is probably the best characterised virus. A very basic question is how TMV interacts with water (and with other liquids). For example, the location of water molecules on the exterior surface and in the tubular channel should depend on temperature and pressure.

Environmental electron microscopy can be used to observe wetting scenarios below 50 nm, on single virions. The condensation of droplets, but also the formation of water wedges was detected for virions adsorbed on various solid surfaces. Further characterisation was achieved with scanning force microscopy, and with infrared spectroscopy (also highly localised on 10 nm areas).

However, the big challenge is the molecular scale below 5 nm. As yet, this was merely addressed indirectly, with transmission electron microscopy: Solutions of metal complexes interact with the virus, similar to stains, but with the ability to effuse from the 4 nm channel inside the virion. A potential uses of this setup is highly localised slow drug delivery.

References:
A.A. Khan, E.K. Fox, M.L. Gorzny, E. Nikulina, D.F. Brougham, C. Wege, A.M. Bittner, "pH control of the electrostatic binding of gold and iron oxide nanoparticles to Tobacco Mosaic Virus", Langmuir (2013), in print
J.M. Alonso. T. Ondarçuhu, A.M. Bittner, "Integration of Plant Viruses in Electron Beam Lithography Nanostructures", Nanotechnol. 24 (2013) 105305
W. Nuansing, A. Rebollo, J.M. Mercero, J. Zuniga, A.M. Bittner, "Vibrational spectroscopy of self-assembling aromatic peptide derivates", J. Raman Spectrosc. 43 (2012) 1397-1406
S. Balci, K. Hahn, P. Kopold, A. Kadri, C. Wege, K.

Kern, A.M. Bittner, Nanotechnology 23 (2012) 045603, "3 nm Wide Cobalt-Iron(-Nickel) Wires inside Tobacco Mosaic Virus"
A. Mueller, F.J. Eber, C. Azucena, A. Petershans, A.M. Bittner, H. Gliemann, H. Jeske, C. Wege, ACS Nano 5 (2011) 4512-4520, "Inducible Site-Selective Bottom-Up Assembly of Virus-Derived Nanotube Arrays on RNA-Equipped Wafers"
A. Kadri, E. Maiss, N. Amsharov, A.M. Bittner, S. Balci, K. Kern, H. Jeske, C. Wege, Virus Res. 157 (2011), 35-46, "Engineered Tobacco mosaic virus mutants with distinct physical characteristics in planta and enhanced metallization properties"

Keywords: TMV, physics, microscopy, spectroscopy, wetting.

O-86

Self-assembly triggered by self-assembly: virus-like particles loaded with supramolecular nanomaterials

Andrés de la Escosura*[1], Melanie Brasch[2], Jealemy Galindo[2], Eduardo Anaya Plaza[1], Francesca Setaro[1], Daniel Luque[3], José Carrascosa[3], José Caston[3], Jeroen Cornelissen[2], Tomás Torres[1]

[1] Department of Organic Chemistry. Universidad Autónoma de Madrid. [2] Laboratory for Biomolecular Nanotechnology, MESA Institute. University of Twente.Enschede. Netherlands. [3] Department of Structure of Biomolecules. Centro Nacional de Biotecnologia/CSIC . Madrid.

The self-assembly of biomolecules such as the coat proteins (CPs) of virus capsids offer great opportunities in nanotechnology and nanomedicine, leading to monodisperse platforms where different chemical species can be organized through covalent or non-covalent bonding. Yet, because the covalent approach for the modification of virus capsids is still a demanding task, efficient and straightforward supramolecular strategies are highly desirable. The Cowpea Chlorotic Mottle Virus (CCMV), in particular, is a plant virus of 28 nm in diameter with an interesting sensitivity to pH and ionic strength. Depending on these factors, CCMV capsids can rapidly be disassembled *in vitro* into CP dimers and then re-assembled again. In this presentation, we will show several examples of hierarchical and cooperative processes in which self-assembled organic chromophores serve as templates for the assembly of the CCMV CP around them. In such processes, the structure of the self-assembled templates determines the size and geometry of the resulting virus-like particles (VLPs), while confinement within the VLPs also determines the structure of the chromophore self-assemblies. The precise structure and assembly properties of these particles have been studied in detail by microscopy techniques, and sophisticated VLPs have been designed and prepared for multimodal photodynamic therapy (PDT) and imaging.

Publications related to this work:

1. A. de la Escosura, R. Nolte and J. Cornelissen, *J. "Viruses and Protein Cages as Nanocontainers and Nanoreactors". Mat. Chem.* 2009, *19*, 2274
2. A. de la Escosura, P. Janssen, A. Schenning, R. Nolte and J. Cornelissen, "*Encapsulation of DNA-Templated Chromophore Assemblies within Virus Protein Nanotubes*", *Angew. Chem. Int. Ed.* 2010, *49*, 5335
3. M. Brasch, A. de la Escosura, Y. Ma, A. Heck, T.

Torres and J. Cornelissen, *J.* " *Encapsulation of Phthalocyanine Supramolecular Stacks into Virus-Like Particles*" , *Am. Chem. Soc.* 2011, *133*, 6878
4. J. Galindo, M. Brasch, E. Anaya, A. de la Escosura,et al." *Self-Assembly Triggered by Self-Assembly: Protein Cage Encapsulated Micelles as MRI Contrast Agents* " , *Manuscript in preparation.*
5. D. Luque**,** A. de la Escosura, et al." *Structure and Assembly Properties of Phthalocyanine-Loaded Virus-Like Particles*" , *Manuscript in preparation*

Keywords: CCMV, nanotechnology and nanomedicine, chromophores self-assembly, virus-like particles.

SESIÓN PARALELA XI

HIV and other human retroviruses

CHAIRS:
Juan Francisco Lorenzo
María Ángeles Muñoz

O-87

Efficient anti-HIV-1 RNA aptamer obtained by the combination of *in vitro* and *in silico* approaches

Alfredo Berzal-Herranz*[1], Francisco J. Sánchez-Luque[1], Michael Stich[2], Susanna Manrubia[2], Carlos Briones[2,3]

[1] Departamento de Biología Molecular. Instituto de Parasitología y Biomedicina "López-Neyra", CSIC. Armilla Granada. [2] Departamento de Evolución Molecular. Centro de Astrobiología, CAB/CSIC-INTA. Torrejón de Ardoz, Madrid [3] Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas [CIBERehd].

Human Immunodeficiency Virus type-1 (HIV-1) is a lentivirus belonging to the family *Retroviridae*. Its high mutation rate determines the quasispecies structure of the viral population, which, together with its recombination rate, results in the quick selection of drug-resistant variants and the evasion of the host's immune system. HIV-1 genome is composed of two copies of a single stranded, 9.2 Kb-long RNA molecule containing multiple and highly conserved structural RNA domains, which play key roles in essential viral processes. Interference with these RNA domains by either disrupting their structure or blocking their interaction with viral or cellular factors, may seriously compromise HIV-1 viability. Thus, such HIV-1 RNA domains (especially abundant at the 5'-untranslated region, 5'UTR, of its genome[1,2]) have revealed as potential antiviral targets. RNA aptamers are among the most promising tools to specifically bind to HIV-1 structural RNA domains[3,4]. They are selected *in vitro* by the so-called SELEX (Systematic Evolution of Ligands by Exponential Enrichment) procedure[5,6]. However, this method imposes a minimal length to the resulting molecules, incompatible with a large-scale chemical production. Aptamer shortening up to its minimal active domain is usually accomplished by very time-consuming, trial-and-error approaches[7].

We have performed a SELEX procedure using the entire HIV-1 5'UTR as the target molecule. After 14 amplification-selection rounds, a collection of 64 nt-long RNA aptamers was obtained, some of which were able to specifically bind to any of the functional domains present in the complete 5'UTR. Selected sequences and structures were subjected to an innovative *in silico* analysis that allowed us the identification of a highly conserved 16 nt-long RNA hairpin motif containing a common 8 nt-long hairpin loop. Based on this result, an *in silico*-engineered molecule termed RNA16(+) was synthesized, which specifically and efficiently bound to the 5'UTR of the genomic RNA *in vitro*. Further analyses demonstrated that the use of RNA16(+) resulted in inhibitions higher than 60% in the HIV-1 production in a human cell line.

[1]Berkhout B 1996. Prog Nucleic Acid Res Mol Biol 54:1
[2]Kuiken C, et al. 2011. HIV Sequence Compendium 2011
[3]Ducongé F, Toulmé JJ. 1999. RNA 5:1605
[4]Kolb G, et al. 2006. RNA Biol 3:150
[5]Tuerk C, Gold L. 1990. Science 249:505
[6]Ellington AD, Szostak JW. 1990. Nature 346:818
[7]Berezhnow A, et al. 2012. Mol Ther 20:1242

Keywords: aptamers, anti-HIV RNAs, RNA structure/function, 5'UTR HIV.

O-88

Interaction between HIV PAMPs and the RIG-I pathway

Elisa de Castro Álvarez*[1], Marcel Ooms[1], Viviana Simon[1,2], Adolfo García Sastre[1,2]

[1] Department of Microbiology. Icahn School of Medicine at Mount Sinai. New York. USA [2] Department of Medicine, Division of Infectious Diseases. Icahn School of Medicine at Mount Sinai. New York, USA.

The success of the immediate innate immune response relies on the recognition of conserved structures, termed pathogen associated molecular patterns (PAMPs), specifically present in pathogens but not in the host. Microbial and viral PAMPs are detected by pattern recognition receptors (PRRs) such as TLRs (Toll-like Receptors), NLRs (NOD-like Receptors), RLH (RIG-I-like helicases), as well as cytoplasmic DNA sensing pathways. PAMPs initiate the induction of several intracellular signaling events, including activation of the NF B and IRF pathways, and the inflammasome. The potent, but short-lived activation of these innate response pathways triggers the induction of cytokines and interferons, which restricts the pathogen infection. Additionally, induction of the innate immune system is required for activation of long-lived adaptive immune responses. Interestingly, many viruses have adapted to the presence of an innate immune system by specifically counteracting critical components of these danger pathways.

The RLRs RIG-I and melanoma differentiation-associated gene 5 (MDA5) are DExD/H box RNA helicases that can detect viral RNA in the cytoplasm of infected cells. Potential PAMPs that could be present in HIV are single-stranded structured RNA and cytoplasmic viral double stranded DNA and DNA/RNA intermediates. Although HIV does not seem to potently activate the innate immune system, our knowledge about potential HIV PAMPs is still very limited.

To determine whether HIV contains PAMPs, we extracted viral RNA from HIV-1 virions, which was able to efficiently induce interferon upon transfection in A549 cells. Subsequent experiments with HIV mutants that were unable to package viral RNA or perform reverse transcription, indicated that viral RNA and early reverse transcription products were both able to induce interferon. Phosphatase treatment of viral RNA and RIG-I knockdown completely abrogated interferon induction indicating that phosphorylated viral RNA is detected by RIG-I leading to induction of IFN beta.

In conclusion, our data indicate that HIV contains potential PAMPs. We are currently investigating whether these PAMPs are also detected during HIV infection or counteracted by HIV itself.

Keywords: RIG-I, HIV RNA, innate immune signaling.

O-89

Improving adaptive and memory immune responses of an HIV/AIDS vaccine candidate MVA-B by deletion of vaccinia virus genes (C6L and K7R) blocking interferon signaling pathways

Juan García Arriaza*[1], Pilar Arnáez[1], Carmen E. Gómez[1], Carlos Óscar S. Sorzano[2], Mariano Esteban[1]

(1) Department of Molecular and Cellular Biology. Centro Nacional de Biotecnología. Consejo Superior de Investigaciones Científicas (CSIC). Madrid (2) Biocomputing Unit. Centro Nacional de Biotecnología. Consejo Superior de Investigaciones Científicas (CSIC) Madrid.

Poxvirus vector Modified Vaccinia Virus Ankara (MVA) expressing HIV-1 Env, Gag, Pol and Nef antigens from clade B (termed MVA-B) is a promising HIV/AIDS vaccine candidate, as confirmed from results obtained in a prophylactic phase I clinical trial in humans. To improve the immunogenicity elicited by MVA-B we have generated and characterized the innate immune sensing and the *in vivo* immunogenicity profile of a vector with a double deletion in two vaccinia virus (VACV) genes (C6L and K7R) coding for inhibitors of interferon (IFN) signaling pathways. The innate immune signals elicited by MVA-B deletion mutants (MVA-B deltaC6L and MVA-B deltaC6L/K7R) in human macrophages and monocyte-derived dendritic cells (moDCs) showed an up-regulation of the expression of IFN- , IFN- / -inducible genes, TNF- , and other cytokines and chemokines. A DNA prime/MVA boost immunization protocol in mice revealed that these MVA-B deletion mutants were able to improve the magnitude and quality of HIV-1-specific CD4+ and CD8+ T cell adaptive and memory immune responses, which were mostly mediated by CD8+ T cells of an effector phenotype, with MVA-B deltaC6L/K7R being the most immunogenic virus recombinant. CD4+ T cell responses were mainly directed against Env, while GPN-specific CD8+ T cell responses were induced preferentially by the MVA-B deletion mutants. Furthermore, antibody levels to Env in the memory phase were enhanced by the MVA-B deletion mutants compared to the parental MVA-B. These findings revealed that double deletion of VACV genes that act blocking intracellularly the IFN signaling pathway confers an immunological benefit, inducing innate immune responses and increases in the magnitude, quality and durability of the HIV-1-specific T cell immune responses. Our observations highlighted the immunomodulatory role of the VACV genes C6L and K7R, and that targeting common pathways, like IRF3/IFN- signaling, could be a general strategy to improve the immunogenicity of poxvirus-based vaccine candidates.

Keywords: MVA, poxviruses, HIV/AIDS vaccine, immunomodulatory vaccinia genes,immune response, prime-boost,immunogenicity, mice.

O-90

Cleavage sites variability across human immunodeficiency virus type 1 variants

Esther Torrecilla[1], Teresa Llácer[1], Patricia Álvarez[1], África Holguín*[1]

[1] Laboratorio de Epidemiología Molecular del VIH-1, Servicio de Microbiología y Parasitología. IRYCIS . Hospital Universitario Ramón y Cajal y CIBER-ESP, Madrid.

Background: HIV-1 sequences available at GenBank allow us to study the viral conserved motives in each viral protein. Protease (PR) cleavages viral polyproteins at cleavage sites (CS) to form Gag proteins (matrix: P17, capsid: P24, nucleocapsid: P7, P6 and two spacer peptides: P1 and P2) and Pol (PR, reverse transcriptase (RT) and integrase (INT)). Polymorphisms at CS can influence processing, viral fitness and the virological outcome of specific antiretroviral regimens. Scarce data of HIV-1 variant-associated CS variability is available.

Objective and methods: The effect of HIV-1 variants on CS variability was examined using all *gag* and *pol* HIV-1 sequences deposited in GenBank, focusing on the 110 residues (10 per site) involved at 11 CS: P17/P24, P24/P2, P2/P7, P7/P1, P1/P6*gag*, NC/TFP, TFP/P6*pol*, P6*pol*/PR, PR/RT_{P51}, RT_{P51}/RT_{P66}, RT_{P66}/INT. CS consensus sequences for each HIV-1 groups, subtypes, circulating and unique recombinant forms (CRF, URF) and simian immunodeficiency virus from chimpanzees (SIVcpz), were inferred from our alignments and compared to those reported by GenBank. We grouped CRF sharing similar recombination patterns in 12 recombinant families. Conservation rate and less conserved residues at each CS were also identified across all HIV-1 variants.

Results: A total of 9,052 *gag* and 3,914 *pol* sequences were downloaded from GenBank. They included 43/43, 7,913/3,269, 1,060/566, 12/11 and 24/25 *gag/pol* sequences ascribed to HIV-1 groups (O, N, P), 9 group M subtypes, 51 CRF, URF and SIVcpz available at GenBank. The residue conservation in our alignments was compared to the inferred consensus sequence for each variant and to the HIV-1 consensus of consensus sequence provided by GenBank. In all variants, the most conserved CS were PR/RT_{P51} (86%), RT_{P51}/RT_{P66} (85%) and P24/P2 (79%) and the less P2/P7 (38%). P2/P7 showed different lengths across variants. Overall, recombinants presented a higher CS conservation than subtypes (83% *vs.* 68%, $p<0.001$), presenting the highest CS variability the AG recombinant family (68%) and subtypes F1, G and B (all less than 53%). The most variable residue at each CS was also identified in each subtype and recombinant family across all downloaded sequences.

Conclusion: This study firstly describes the cleavage site conservation degree at amino acid level across HIV-1 variants and sites in a large sequence dataset. However, the biological significance of the found HIV-1 variant-associated variability in each processing site needs to be further investigated.

Keywords: HIV, genetic variability, cleavage sites, gag, pol, subtypes, recombinants.

O-91

Changes in codon-pair bias of human immunodeficiency virus type 1 has profound effects on virus replication in cell culture

Gloria Martrus[1], Cristina Andrés[1], Mariona Parera[1], María Nevot[1], Bonaventura Clotet[1], Miguel Ángel Martínez*[1]

(1) IrsiCaixa. Hospital Germans Trias i Pujol. Badalona.

Human immunodeficiency virus type 1 (HIV-1) has biased nucleotide compositions different from human genes. This raises the question of how evolution has chosen the nucleotide sequence HIV-1 observed today, or to what extent the actual encoding contributes to virus replication capacity, evolvability and pathogenesis. Here, we applied the previously described synthetic attenuated virus engineering (SAVE) approach to HIV-1. Using synonymous codon pairs, we rationally recoded and codon pair–reoptimized and deoptimized different moieties of the HIV-1 gag and pol genes. Deoptimized viruses had significantly lower viral replication capacity in MT-4 and peripheral blood mononuclear cells (PBMCs). Varying degrees of ex vivo attenuation were obtained, depending upon both the specific deoptimized region and the number of deoptimized codons. Reoptimized viruses carrying a large number of synonymous mutations were not attenuated neither were more virulent than the wild type virus. These data demonstrate that synthetic synonymous codon pair reengineering is a useful strategy to phenotypically affect the replicative properties of HIV-1.

Keywords: HIV-1, codon-pair bias, deoptimization, viral replication in culture.

O-92

Lost opportunities: new diagnoses of HIV infected children in Spain between 2005-2011

Maria Luisa Navarro*[1], Talia Sainz[2], María Isabel González Tomé[3], Santiago Jiménez de Ory[2], Pere Soler Palacín[4], María Espiau[4], Grupo de Trabajo CoRISpe[5]

(1) Sección de Enfermedades Infecciosas. Servicio de Pediatría. Hospital General Universitario Gregorio Marañón. Madrid (2) Laboratorio de Inmunobiología Molecular. Hospital General Universitario Gregorio Marañón.Madrid (3) Sección de Inmunodeficiencias. Servicio de Pediatría. Hospital 12 de Octubre. Madrid (4) Unitat de Patología Infecciosa i Immunodeficiències de Pediatria. Hospital Universitari Vall d` Hebron. Barcelona (5) Grupo Trabajo. CoRISpe. Spain.

Background: Public health initiatives aiming to prevent vertical transmission of HIV infection led in the last few years to a dramatic decline in the incidence of HIV infection among children in high-income countries. The objective of this study is to analyze the main characteristics of the few pediatric cases diagnosed in Spain in the last seven years.

Methods: Cross-sectional study including new diagnoses of HIV infection between January 2005 and December 2011. Demographic, clinical and inmunovirologic data were collected retrospectively from the CoRISpe Database, which includes all children living with HIV (CLHIV) in Spain.

Results The Spanish Cohort of HIV-infected Children (CoRISpe) includes to date 925 CLHIV, of whom 171 were diagnosed during the study period. 86% of cases corresponded to vertical transmissions (51.7% female). Eighty-seven children (59%) were born in Spain, of them 51.7% were born to a Spanish mother, followed by 21.8% born to a sub-Saharan mother. Regarding children born in Spain, data about perinatal conditions were available for 77 patients: 29.9% of mothers were diagnosed before pregnancy, 23.4% during pregnancy, 10.4% during labour and 36.4% after delivery. Median age of the children born in Spain at the moment of diagnosis was 0.4 years (IQR: 0.1-1.6), while it was 3.3 years (IQR: 2-6.4) for children born abroad ($p<0.001$). Median CD4/mm^3 was 1403 (IQR: 691-2749) and 840 CD4/mm^3 (IQR: 432-1274) respectively, and CD4%: 27 (IQR: 19-45) and 20 (IQR: 16-27.8), respectively (all $p<0.001$).

Conclusions: Despite widespread routine testing and the efficacy of specific interventions to avoid mother-to-child transmission, there are ongoing new diagnoses of HIV infection among children in our country. CLHIV born abroad are diagnosed late, and accordingly show a worse immunological condition. Up to 59,2% of the new diagnoses performed during the study period correspond to children born in Spain, representing lost opportunities to avoid HIV infection, mainly due to delayed maternal diagnosis or failure to implement prophylaxis of vertical transmission.

Keywords: HIV, paediatric, epidemiology, cohorts, vertical transmission.

O-93

Glyco and phosphorous decorated dendrimers as new tools in the search for effective anti-HIV DC-based immunotherapies

Enrique Vacas Córdoba*[1], Hugo Bastida[1], Hartmut Komber[2], Dietmar Appelhans[3], Anne M Caminade[4], Jean P Majoral[4], Rafael Gómez[4], Francisco J. de la Mata[5], Marjorie Pion[1], María Ángeles Muñoz Fernández[1]

(1) Laboratorio Inmunobiología Molecular. Hospital General Universitario Gregorio Marañón. Madrid (2) Department of Analytics. Leibniz-Institut für Polymerforschung Dresden e.V. Dresden, Germany (3) Department of Polymer Structures. Leibniz-Institut für Polymerforschung Dresden e. V. Dresden, Germany (4) Dendrimères et Hétérochimie. Laboratoire de chimie de coordinatíon du CNRS. Tolousse. France (5) Inorganic Chemistry Department. UAH. Alcalá de Henares, Madrid.

Dendritic cells (DC), which play a major role in development of cell-mediated immunity, represent opportunities to develop novel anti-HIV vaccines. Dendrimers have been proposed as new carriers to ameliorate DC antigen loading and in this way,

the potential use of maltose decorated glycodendrimers as well as cationic phosphorus dendrimers have been determined. Thus, immunostimulatory properties of these dendrimers on human DC were evaluated in the context of HIV infection.

We have demonstrated that DC treated with glycodendrimers were fully functional with respect to viability and maturation. Moreover, positively charged glycodendrimers in association with HIV-derived peptides were able to increase the uptake of these peptides into DCs in comparison with HIV-derived peptides alone. Nevertheless, iDC and mDC phenotypes as well as mDC functions such as migration ability and cytokines profile production were changed, showing a more activated immuno profile. These results showed the potential carrier properties of dendrimers with sugar moieties to activate the immune system by the way of DCs stimulation.

On the other hand, phosphorus dendrimers showed ability to deliver HIV-derived peptides in DC, but they induced important changes in phenotype. Moreover, the treatment of mDC with the second generation phosphorous dendrimer and derivated dendriplexes modified cellular migratory properties, altered their capacity to stimulate allogenic naïve T cells *in vitro* and impeded the production of pro-inflammatory cytokines, suggesting a tolerogenic profile of treated DC. Thus, phosphorus dendrimers could not be used as vaccines because they would not have the ability to induce an immune response.

Summing up, our results showed the potential carrier properties of glycodendrimers to activate the immune system by the way of DC stimulation. In case of phosphorous dendrimers, although they were not able to induce immune response, our data suggest that there are at least three potential therapeutic applications where these phosphorus dendrimers could be tested: i) topic anti-inflammatory agents ii) compounds for allograft rejection or autoimmune diseases; iii) induction of specific tolerance with antigen-loaded DC against allergy reaction for example.

Keywords: HIV vaccine, dendritic cells, glicodendrimer, phosphorous dendrimer.

SESIÓN PARALELA XII

Plant viruses

CHAIRS:
José Antonio Daròs
Javier Romero

O-94

Environmental effect on life-history traits associated with virus-tolerance in *Arabidopsis thaliana*

Jean Michel Hily*[(1)], Fernando García Arenal[(1)]

(1) Centro de Biotecnología y Genómica de Plantas UPM-INIA y E.T.S.I Agrónomos. Universidad Politécnica de Madrid .

Plants have developed a variety of mechanisms to compensate for the cost of biotic and/or abiotic stresses. Upon parasite infection, host may respond differently, which may be categorized into different strategies [1]. In one of the tactic, host can go through life-history modifications that compensate for the negative effects of parasitism, which is considered part of tolerance mechanisms. Life-history theory makes predictions for the adjustment of resource investment by organisms, based on the notion that trade-offs exist between resources allocated to different fitness components: growth, reproduction and survival [2]. Models for evolution of resource allocation predict that parasitized organisms will allocate more resources to reproduction, subtracting them from those dedicated to growth and survival [2].

Our group tested predictions of life-history evolution theory by establishing the plant-parasite system of *Arabidopsis thaliana* and the generalist virus *Cucumber mosaic virus* (CMV) in order to study the effect of virus infection on plant growth and reproductive effort. It was shown that plastic modifications upon CMV infection were substantial compared to mock inoculated plants [3]. More specifically, such modification in allocation of resources due to infection and life-history responses was different depending on the allometric features of *Arabidopsis* genotypes and two groups were distinctively significant, with group1 genotypes being on average more tolerant than those of group2 [3].

Since tolerance is a quantitative trait based on phenotypic plasticity upon infection, the optimal amount of resources allocated to each of these components may be corrected according to environmental conditions in order to maximize the organism's fitness. Hence, tolerance might be environment-dependent. To test this hypothesis, four *Arabidopsis* ecotypes were inoculated with CMV and grown under different light and temperature conditions. The outcome of the plants-virus interaction varied from high virulence to tolerance within the range of conditions tested. Nonetheless, phenotypes were genetically determined. The most representative of many changes observed in the phenologic and phenotypic traits tested will be presented to illustrate this major result.

1- Agnew P et al. (2000), Microbes Infect 2: 891–896
2-Stearns SC (1976), Q Rev Biol 51: 3–47
3-Pagan I et al.2008), Plos Pathogens 4: 8: e1000124

Keywords: plant, virus-tolerance, environment.

O-95

Role of the crinivurus tomato chlorosis virus P22 supressor of RNA silencing during the viral infection process

Yazmín Landeo Ríos*[1], Jesús Navas Castillo[1], Enrique Moriones [1], Carmen Cañizares[1]

(1) Department of Plant-pathogen interactions. Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" (IHSM-UMA-CSIC). Málaga.

Post-transcriptional gene silencing (PTGS) is an antiviral defence mechanism in plants, which can be suppressed by virus-encoded suppressors of silencing. Tomato chlorosis virus (ToCV, genus *Crinivirus,* family *Closteroviridae*) is a plant virus with a large bipartite, single-stranded, positive-sense RNA genome that encodes several RNA silencing suppressors. The mode of action of one of them, the RNA1 encoded p22 suppressor, has been extensively studied as an isolated protein. However, to understand the role of the p22 viral suppressor under the viral context, a suppressor-deficient mutant virus is needed. For this, we constructed a mutant virus that fails to express p22 (ToCV p22 RNA1). The dynamics of the infection process were compared for the wild-type ToCV and the p22-deletion mutant either in wild-type *Nicotiana benthamiana* (Nb) or in *N. benthamiana* rdr6i-Nb plants that lack the RNA-dependent RNA polymerase 6 (RDR6), one of the key components of diverse RNA silencing pathways. Our agroinfiltration results show that, in the absence of p22 expression, ToCV p22 RNA1 accumulation is not altered, indicating that p22 is not required for RNA1 self-replication. In contrast, ToCV p22 RNA1 does not efficiently support the trans accumulation of ToCV RNA2. Interestingly, our results also show a more efficient systemic infection ability of the p22-deletion mutant virus in rdr6i-Nb plants than in wild-type Nb plants, linking p22 function with the RDR6 pathway.

Keywords: *Closteroviridae,* crinivirus, gene silencing suppressor, tomato chlorosis virus

O-96

Identification of pathogenicity determinants involved in the adaptation of Plum pox virus strain C (PPV-C) to its natural host, *Prunus avium*

María Calvo*[1], Tadeusz Malinowski[2], Juan Antonio García[1]

(1) Departamento de Genética Molecular de Plantas. Centro Nacional de Biotecnología-CSIC. Madrid
(2) Department of Plant Physiology and Biochemistry. Research Institute of Horticulture. Skierniewice. Poland.

PPV is the causing agent of the Sharka disease in fruit trees of the genus *Prunus*. So far, several PPV

strains have been identified in the field. Among these strains, PPV-C, which is one of the less common, is supposed to specifically infect cherry trees in nature.

Making use of a PPV-C isolate (SwCM) that had been adapted to *Nicotiana* species and a PPV-C isolate (BY101), which had been directly isolated from *Prunus avium*, we have generated two infective full-length cDNA clones (pIC-PPV-SwCM and pIC-PPV-SwCBY101) that display different pathogenicity features in several hosts. Taking advantage of classical cloning and DNA shuffling techniques, we have constructed a set of chimeras in order to determine which viral factors are involved in the specific adaptation to *P. avium* and *Nicotiana benthamiana*.

Our results indicate that PPV-C is better adapted to *P. avium* than to other *Prunus* species, such as *P. persica* or *P. domestica*. The region responsible for specific systemic movement in *P. avium* and *N. benthamiana*maps between nucleotides 2940 and 4574 of the PPV genome, which covers the C- terminal P3 (+PIPO), 6K1 and the N- terminal CI coding sequences.

According to our results, a single amino acid change in 6K1 (T/P, BY101/SwCM) is enough for the virus to adapt to each host. Interestingly, this residue is located at the -6 position of the NIa-Pro recognition sequence between 6K1 and CI. This suggests that the processing of the 6K1-CI junction could depend on an interaction between NIa-Pro and an unknown specific host factor, which would determine viral fitness in each host.

Keywords: plum pox virus, prunus, pathogenicity determinants.

O-97

A plant virus infection improves host water stress tolerance by modulating the transcription activity of a bHLH transcription factor

Vicente Pallas[1], Frederic Aparicio*[1]

(1) Departamento de Virología Molecular de Plantas. IBMCP (CSIC-UPV) Valencia.

Plant-virus interactions affect host metabolism and physiology patterns. In some cases these effects are triggered by direct interactions between viral and host factors including transcription regulators which can affect the regulation of many cellular pathways. One of the plant viral proteins for which a large number of host interactions have been identified is the coat protein (CP). Viral CPs are multifunctional proteins playing major roles in most of the virus infection steps. In this sense the CP of Alfalfa mosaic virus (AMV) (*Bromoviridae* family) accumulates at the nucleolus and is involved in the regulation of replication and translation of viral RNAs, in cell-to-cell and systemic movement of the virus and in virion formation. AMV genome consists of three single-stranded RNAs of plus sense polarity. RNAs 1 and 2 encode the replicase subunits P1 and P2, respectively, whereas RNA 3 encodes the movement protein and serves as a template for the synthesis of the non-replicating subgenomic RNA4 (sgRNA4), which encodes the CP. ILR3 is a transcription factor belonging to the basic helix–loop–helix (bHLH) family of transcrip-

tion regulators. Plant bHLH proteins have been predicted to be implicated in plant metabolism and development. A previous study has shown that, in arabidopsis, ILR3 would act repressing expression, among others, of a NEET protein (atNEET; at5g51720). The NEET family is a group of proteins involved in diverse biological processes, as autophagy, apoptosis, aging, diabetes, and reactive oxygen homeostasis. They form a novel structure, the NEET fold, in which two promoters intertwine to form a unique redox-active labile 2Fe-2S cluster binding domain. AtNEET has a critical participation in diverse plant processes as plant development, senescence, ROS homeostasis and iron metabolism. Moreover, RNAi interference lines with diminished levels of atNEET mRNA were more resistant to salinity and osmotic stresses.

Here we show that the AMV CP directly interacts with ILR3 from both Arabidopsis and *Nicotiana tabacum*. Moreover, ILR3-CP interaction seems to enhance ILR3 repression activity since mRNA level of NEETis down-regulated in AMV infected plants. Interestingly, these infected plants also showed significant improved tolerance to salinity and water deficiency conditions. These results suggest that AMV affects plant metabolism to help growth on deficient water and soil conditions extending host survival under these abiotic stresses.

Keywords: plant virus-interactions; Alfamovirus.

O-98

Allosteric regulation of P1 protease activity could modulate potyviral replication

Fabio Pasin*[1], Carmen Simón Mateo[1], Juan Antonio García[1]

(1) Departamento de Genética Molecular de Plantas. Centro Nacional de Biotecnología. Madrid.

Viruses are obligate parasites that hijack host cellular components to assure their replication and propagation. To overcome the limited coding capacity of their genome, the synthesis of large polyproteins is a translational strategy used in many RNA viruses. For the polyprotein processing and the release of mature subunits, viruses rely on both cellular and pathogen encoded proteases. To successfully regulate replication, assembly and spreading stages, cleavage kinetics can depend on the endopeptidases processivity, different affinity with the specific cleavage sites, availability of defined cofactors and structural rearrangements that modulate the substrate accessibility.

Members of genus *Potyvirus* (family *Potyviridae*) represent one of the largest group of plant-infecting RNA viruses and belong to the picorna-like supergroup. Potyviruses are characterized by a single-stranded RNA genome further translated into a single large polyprotein. P1 is a serine protease located at the N-terminal end of the polyprotein and once activated by a host factor, it acts in *cis* on its C-terminal releasing itself from HCPro, the second mature protein encoded by the genome. Using *Plum pox virus* as a model, we demonstrate

that although a major role of P1 is not directly related with RNA silencing suppression (as previously suggested), P1 can indeed modulate HCPro function by its self-cleavage activity. Bioinformatic analysis and *in vitro* experiments were performed to get further insights into P1 protease regulation. Apart from defining the boundaries of the C-terminal catalytic domain of P1, we show that the hyper-variable region that precedes the protease domain is predicted as intrinsically disordered and that, in *in vitro* cleavage assays, it acts as antagonist of P1 proteolytic activity. In viral infections, the removal of N-terminal negative regulator of P1 protease is characterized by enhancement of symptom severity and reduction of viral loads. This negative correlation is associated to the over-accumulation of host PR-2 proteins, a known marker of systemic acquired resistance. Relying on these results, we propose that an allosteric mechanism might regulate P1 self-cleavage in order to attenuate viral virulence and relieve the host antiviral response.

Keywords: serine endopeptidase, allostery, virulence, SAR, PPV

O-99

Visual tracking of plant virus infection dynamics using a reporter that activates anthocyanin biosynthesis

Fernando Martínez[1], Leonor C. Bedoya[1], Eszter Majer*[1], David Ortiz[1], Diego Orzáez[1], José Antonio Daròs[1]

(1) Instituto de Biología Molecular y Celular de Plantas. CSIC Universidad Politécnica de Valencia.

Transcription factor Rosea1 (Ros1) from Antirrhinum majus is a 25.7 kDa protein involved in activation of anthocyanin biosynthetic genes. Anthocyanins are the compounds responsible of many of the bright colors of fruits and flowers in plants. We have explored the use of Ros1 as a visual reporter to follow the dynamics of viral infection throughout the host plant. We inserted the Ros1 cDNA in two different positions in the genome of Tobacco etch virus (TEV; genus Potyvirus, family Potyviridae) and we inoculated tobacco (Nicotiana tabacum) plants. Inoculated plants became infected and tissues showing symptoms turned bright red with an about two day delay after symptoms. Red color of infected tissues was clearly visible through naked eyes in real time with no need of specialized instrumentation. RT-PCR analyses of RNA preparations from inoculated plants confirmed that only the red tissues from infected tobaccos contained the virus. Analysis of a series of Ros1-tagged TEV mutants displaying defects in viral accumulation demonstrated a correlation between viral load and an-

thocyanin accumulation in infected tissues. The marker, therefore, informs about viral accumulation qualitatively through eye inspection or quantitatively through a very simple anthocyanin extraction followed by a colorimetric measurement. Serial passages of TEV-Ros1 from plant to plant demonstrated the high stability of the reporter marker in the viral genome. We studied the applicability of this reporter strategy to other virus-host plant combinations, obtaining also good performance for TEV infecting tomato and Nicotiana benthamiana, Turnip mosaic virus (TuMV; family Potyviridae) infecting Arabidopsis thaliana and Tobacco mosaic virus (TMV; family Virgaviridae) infecting N. benthamiana. To improve the performance of this reporter marker, we are currently investigating the possibility of reducing the size of the marker protein and the possibility of fusing it to viral proteins. We are also evaluating other transcription factors from the anthocyanin pathway homologous to Ros1. But in conclusion, our work shows that Ros1 is an alternative to fluorescent proteins as a reporter marker to track virus infection and movement throughout the plant.

Keywords: plant virus, RNA virus, virus movement.

PÓSTERS

P-100

Adeno-associated virus vectors expressing RIG-I-like receptor signaling pathway activating elements as an alternative to recombinant type-I IFNs treatment

Estanislao Nistal Villán*[1], Estefanía Rodríguez García[1], Marianna Di Scala[1], Roberto Ferrero Laborda[1], Gloria González Aseguinolaza[1]

(1) Dpto. de Terapia Génica y Hepatología. Fundación para la Investigación Médica Aplicada. Pamplona.

Detection of pathogens by cells is a key event of defense against infections. RIG-I like receptors (RLRs) detect specific RNAs produced by virus replication and activate a signaling cascade (RLR cascade) that results in the production of interferon beta (IFN-) as well as several other antiviral and proinflammatory genes. Conventional type I IFN treatments are based on administration of recombinant purified protein or administration of different vectors that can produce IFN. Despite its proven antiviral and antitumoral effects, many individuals do not respond to administration of such type I IFNs. This is particularly relevant in the treatment of patients with chronic hepatic infections and those with certain type of tumors. We hypothesize that triggering of RLR pathways instead of direct IFN administration is a valid alternative for the induction of antiviral, antiproliferative and proinflammatory genes. We have developed adeno-associated virus (AAV) vectors expressing different elements of the RLR dependent pathway. Those vectors have been tested for their ability to induce IFN- as well as interferon stimulated and IFN signaling independent genes in tissue culture cells as well as in mice. Preliminary observations show a potent activation of proinflammatory and antiviral programs. We have observed a broad spectrum of cells from different animals as well as different mouse, hamster, rabbit, woodchuck and human tumor model cell lines whose RLR pathway can be triggered by our constructs. We propose the employment of our strategy as an alternative of malignancies currently treated with recombinant type I IFN as well as those that are refractory to such type I IFN treatment. Such strategy can be of particular interest for the treatment of IFN resistant chronic viral infections as well as some neoplasias.

Keywords: RIG-I like receptors, interferon, antiviral, immunostimulation.

P-101

Generation and characterisation of influenza virus mutants affected in counteraction of the innate immune response

Juan Ortín*[1], Maite Pérez-Cidoncha[1], Marian J Killip[2], Víctor Asensio[3], José A. Bengoechea[3], Richard E. Randall[2]

(1) Departamento de Biología Molecular y Celular. Centro Nacional de Biotecnología – CSIC. Madrid (2) School of Biology, Centre for Biomolecular Sciences. University of St Andrews. UK (3) Laboratorio Patogénesis Mi-

crobiana. Fundació d'Investigació Sanitària de les Illes Balears (FISIB). Mallorca.

The influenza A viruses counteract the cell innate immune response at several steps, including blocking the RIG I-dependent activation of interferon (IFN) transcription, the IFN-dependent activation of IFN-stimulated genes (ISGs) and the activity of various ISGs. Most of the influenza virus modulation of the IFN response is provided by the multifunctional NS1 protein. Viruses with deletions in this protein are defective in normal cells or animals. In order to understand the role of NS1 in this counteraction, we introduced random mutations in NS segment. Using this strategy, we have obtained a population of 30.000-40.000 different mutant viruses and selected those able to induce IFN expression and to grow efficiently. The sequence analysis of the selected virus clones showed that non-synonymous mutations occurred at many phylogenetically conserved positions within NS1 protein, suggesting that they could be relevant for the mutant phenotype. Most of these mutant viruses showed normal replication kinetics and high titres, confirming that they maintain good replication fitness. In agreement with the screening procedure used, all mutants induced an IFN response stronger than wt virus. Some of the mutants were not only able to induce IFN but were also unable to block downstream IFN effects. Surprisingly, one of the mutants led to an antiviral activity very similar to the one obtained with delNS1 virus, despite it contains a single aminoacid substitution. Alltogether these results support the use of this strategy for the generation and identification of viral mutants affected in IFN counteraction.

Keywords: Influenza, IFN, NS1.

P-102

Design of TGEV derived vectors and antigenic structures to protect against porcine reproductive and respiratory syndrome

Martina Becares*[1], Carlos M. Sánchez[1], Sarhay Ros[1], Luis Enjuanes[1], Sonia Zúñiga[1]

(1) Department of Molecular and Cell Biology. Centro Nacional de Biotecnología, (CNB-CSIC) Madrid.

Current vaccines against Porcine Reproductive and Respiratory Syndrome (PRRSV) have a limited efficacy. Modified live vaccines based on attenuated viruses lead to similar non-effective responses than PRRSV. Therefore, vector-based vaccines could represent and advantage to stimulate both humoral and cell immune responses. Nevertheless, the results reported to date using viral vectors are not fully satisfactory and new vectors must be explored. Transmissible gastroenteritis virus (TGEV) based vectors have the capability of expressing high levels of heterologous genes, are potent interferon-a inducers, and present antigens in mucosal surfaces, eliciting both secretory and systemic immunity. In addition, TGEV based vector vaccines expressing PRRSV antigens represent a promising candidate to provide protection against two porcine viruses: PRRSV and TGEV. A set of coronavirus-derived vectors, based on TGEV was constructed. These vectors expressed PRRSV M protein and GP5 mutants with altered glycosylation patterns, since it has been proposed that removal of the glycosylation sites could lead to the

improvement of the immune response against PRRSV. Vaccinated animals showed a clear humoral response against PRRSV GP5 and M proteins. Nevertheless, the immune response elicited by these vectors did not provide full protection. The partial stability of GP5 protein in TGEV vector, together with a possible induction of negative signals for the immune system may be the cause of the partial protection observed. To improve rTGEV vector stability, the 68 most N-terminal aminoacids of the GP5 protein, covering the ectodomain of the protein that contain the epitopes relevant for protection, were expressed by rTGEV vectors. This protein fragment includes the epitopes eliciting the immune response against GP5 protein, but may lack protein toxic domains or negative signals. After 16 passages in tissue culture, a significant improvement of rTGEV stability was observed, suggesting that reduction of the insert size may be a promising strategy to increase rTGEV stability. Protection conferred by these vectors, alone or in combination is being evaluated.

Keywords: PRRSV, coronavirus, viral vectors, infectious cDNA.

P-103

Reliable determination of the population structure of dividing human lymphocytes

Cristina Peligero*[1], Jordi M. Argilaguet[1], W. Clayton Thompson[2], H. Thomas Banks[2], Andreas Meyerhans[1]

(1) Department of Experimental and Health Sciences. Universitat Pompeu Fabra. Barcelona (2) Center of research in Scientific Computation and Center for Quantitative Sciences in Biomedicine. North Carolina State University. Raleigh, USA.

Lymphocyte proliferation, which follows antigenic stimulus resulting from an invading microorganism, is a hallmark of the adaptive immune system. Studying the quantitative dynamics of lymphocyte proliferation is essential for a better understanding of immune responses. Since it was first described, serial dilution of the fluorescent dye carboxyfluorescein succinimidyl ester (CFSE) has become the almost universal method for the determination of cellular division histories. To obtain dynamic information from CFSE-labelled proliferating cell populations, the fluorescence intensity profiles need to be analysed. Individual peaks can be fit with predefined shapes, usually normal or log-normal distributions, so that cell numbers per generation can be estimated. Therefore, the ability to reliably estimate cell numbers per fluorescence peak depends on clear peak separations that is often not attained in experiments using blood-derived human lymphocytes.

Here, we have developed a mathematical model that predicts the population structure evolution

of human CD4 and CD8 T cells from histograms of time-series measurements of CFSE-labelled, phytohaemagglutinin-stimulated peripheral blood mononuclear cells. It combines (i) the direct analysis of flow cytometry-derived CFSE histograms with (ii) a statistical evaluation of the variability in the histogram data and (iii) a cyton model-based description of cell fate decisions. This model enables to derive biologically meaningful proliferation parameters and to reconstruct the population generation structure for viable cells. As the model is not dependent on clear CFSE peak separations, it is potentially of broad utility for the detailed characterization of the immune cell dynamics from human PBMCs in studies of human infections or vaccinations.

Keywords: T-cell proliferation, CFSE, mathematical modeling.

P-104

Clinical clearance after applying autologous hemolmmunotherapy in a high risk human papilloma virus carrier woman with uterine cervical dysplasia. A case report based on comparative virology

Ricardo A. Roa Castellanos[1]

(1) Faculty of Veterinary. Universidad Complutense de Madrid.

A 31 years old woman, smoker since her 17, mother of a 4 years old child, after presenting 5 months of continuous uterine bleeding is diagnosed, at colposcopy, with inflammatory papilloma. Histopathology evaluation is performed on an abnormal acetic-white epithelium revealing a low grade squamous intraepithelial lesion (LSIL) that concurs with cytopathic changes induced by Human Papilloma Virus (HPV). Two samples were taken for biopsy. PCR –*Amplicor® HPV-* was carried out twice to differentiate diagnosis. Lab results showed Cervical Intraepithelial Neoplasia (CIN)-II and presence of carcinogenic high risk HPV strain(s) endemic for her country (types 16, 18, 31, 33, 35, 39, 58 & 59). Th2 IL-10 has been found increased in women with LSIL[1]. Accordingly, Th1 cytokines known as IL-2 and IL-12 are deficiently produced by cancer and CIN tissues[2]. It is proved that IL-10 is up-regulated in CIN patients. This impaired immune response, created by some viruses (retroviridae and papilloma families) along their physiopathology, seems to promote cancer progression also for other neoplasms. On the other hand, Autologous Hemotherapy (AH) has been useful for integument pathologies such as herpetic infections, atopic dermatitis and scleroderma. It stimulates innate immunity by means of Peripheral Blood Mononuclear Cells (PBMCs) through Th1 eliciting factors such as TNF-alpha currently involved in fibrinolysis. Systemic levels of monocytes, for instance, augment up to 22% during the following week once self blood is injected to patients. After initial 3 administrations, chronic hemorrhage ceased. This therapy was complemented with using of a commercial vaccine. Four months post-treatment initiation, colposcopy and PCR revealed histological normality. PCR developed three times after treatment was negative

even one year after therapy. Its adjusted disease prevalence oscillates around 36,8 by 100.000 inhabitants. Cervical cancer is the first cause of death for women between 25 to 69 years old [3]. Treatment was a exploratory approach, after treating animal papillomas in other species.

[1] Azar, KK et al (2004). Increased Secretion Patterns of IL-10 and TNF-Alpha in Cervical Squamous Intraepithelial Lesions. Human Pathology 35(11): 1376-1384
[2] De GruiJl TD et al. (1999). Differences in cytokine mRNA profiles between premalignant and malignnt lesions of the uterine cérvix. Eur J Cancer. 35: 490-497
[3] De la Hoz, F et al. (2009) Rev Salud Pública 11(3): 454-467

Keywords: HPV clearance, immunotherapy, Th1/Th2 ratio, cervical cancer, precancerous lesions, comparative virology.

P-105

Identification of T cell epitopes from non-structural protein NS1 of bluetongue virus (BTV) serotype 8, capable of cross-reacting with other viral serotypes in mice and sheep

Noemí Sevilla*[1] José Manuel Rojas[1], Verónica Martín[1].

(1) Centro de Investigación en Sanidad Animal (CISA-INIA). INIA Valdeolmos, Madrid.

Bluetongue virus (BTV), an economically important *orbivirus* of the *Reoviridae* family, is a non-enveloped, dsRNA virus that causes a haemorrhagic disease mainly in sheep. In order to estimate the importance of T cell responses during BTV infection, it is essential to identify the epitopes targeted by the immune system. We have previously reported the existence of T cell epitopes in mice and sheep from the VP7 core protein of BTV-8. In the present work, we selected potential T cell epitopes for the C57BL/6 mouse strain from the BTV-8 non-structural protein NS1, using $H2^b$-binding predictive algorithms. 3 MHC-class II-binding peptides and 7 MHC-class I binding peptides were selected. Peptide binding assays confirmed all MHC-class I predicted peptides bound MHC-class I molecules. The immunogenicity of these 10 predicted peptides was then determined using splenocytes from BTV-8-inoculated C57BL/6 mice. Three MHC-class I binding peptide consistently elicited peptide-specific IFN- production as measured by ELISPOT assay. Cytotoxic T lymphocytes (CTL) were also generated against these 3 peptides in BTV-8 in-

fected mice. Importantly, these CTL were also able to recognise target cells infected with different BTV serotypes. Flow cytometry analysis using intracellular cytokine staining confirmed that $CD8^+$ T cells mediated the response to these 3 NS1-derived T cell epitopes. Similarly, one MHC-class II peptides was demonstrated to be a CD4+ T cell epitopes using a combination of IFN- ELISPOT, proliferation and flow cytometry assays in BTV-8 infected mice. Notably, two peptides were also consistently immunogenic in sheep infected with BTV-8 using IFN- ELISPOT assays. Both of these peptides appear to stimulate $CD4^+$ T cells. We are currently investigating the ability of these BTV-specific T cells in sheep to respond to different BTV serotypes. The characterisation of these novel T cell epitopes may also provide an opportunity to develop a DIVA-compliant vaccination approach to BTV.

Keywords: bluetongue virus, T cell epitopes.

P-106

Characterization of African swine fever virus (L60/NHV) vaccine strains

Patricia de León*[1], María J. Bustos[1], Ángel L. Carrascosa[1]

(1) Departamento de Virología y Microbiología. Centro de Biología Molecular Severo Ochoa (CSIC-UAM) Madrid.

The generation of protective immunity in pigs inoculated with the ASFV-NHV vaccine strain against both the virulent L60 isolate (of the same genotype) and also against virulent viruses of different genotypes, has been observed in several preliminary experiments. To further analyze the differences in the genome sequences and in the cytokine profile induced by both ASFV strains, a deeper characterization of the L60/NHV stocks was required.

Two fast and specific assays were designed to discriminate between both L60 and NHV genotypes, based on differences in the sequence of EP153R and EP402R ASFV genes. By the use of these assays we were able to detect the presence of NHV genotypes in several previously-considered L60 samples, an "emergence" that was highlighted by serial passages of the original L60 samples on COS cell cultures, but was hardly observed in serial passages on swine macrophages. Several pure L60 clones were obtained by plaque purification on COS cells, that were unable to develop the NHV "contamination" even after 5 successive passages on COS cells, indicating that the L60 genotype was stable on serial passages. Conversion of L60 to NHV genotype was discarded because of their molecular complexity (one A residue in a fixed position in the EP153R gene should be deleted, and a fragment of 87 bp must be acquired by L60 in a specific position of the EP402R gene, to obtain the sequence present in the NHV genotype, and this sequence is not present in the L60 genome), pointing to the most likely possibility that many (if not all) of the available L60 stocks might be "contaminated" with NHV virus.

With the purified stocks of L60 and NHV samples, we have started the analysis of the cytokine profiles induced by both viruses in porcine cells. First,

several cell lines or primary porcine cells were compared for their possibility to be infected by a number of ASFV isolates, and their ability to express different cytokines that could be related with the immune response and the virulence of the virus isolate. The WSL established cell line was selected to perform the RT-PCR analysis of different cytokines (IFNa and b, TNFa, IL1b, IL6, IL12, IL15 and TGFb) induced after infection with the L60/NHV vaccine strains, among other virulent or attenuated ASFV isolates. The possibility to establish a common "virulence profile" with selected cytokines will be discussed.

Keywords: african swine fever virus, vaccine strains, L60/NHV isolates.

P-107

Characterization of an antigenic site on the pandemic 2009 H1N1 influenza virus hemagglutinin which is recognized by murine monoclonal antibodies and relevant for the human antibody response

Blanca García Barreno[1], Teresa Delgado[1], Sonia Benito[1], Inmaculada Casas[2], Francisco Pozo[2], María T. Cuevas[2], Vicente Mas[1], Alfonsina Trento[1], Ariel Rodriguez[3], Ana Falcón[3], Juan Ortín[3], Amelia Nieto[3], José A. Melero*[1]

[1] Departamento de Biología Viral. Centro Nacional de Microbiología and CIBERES.- ISCIII. Madrid [2] Departamento de Virus Respiratorios y Gripe. Centro Nacional de Microbiología and CIBERES.- ISCIII. Madrid [3] Departamento de Biología Celular y Molecular. Centro Nacional de Biotecnología and CIBERES.- CSIC. Madrid.

Four hybridomas producing neutralizing monoclonal antibodies (MAbs) specific for the pandemic influenza virus A/Cal/07/09 hemagglutinin (HA) were isolated from mice immunized with purified virus. Isotyping, competitive binding and reactivity with certain mutant viruses indicated that the four antibodies recognize at least two different but highly overlapping epitopes that are conserved in the HA of Spanish pandemic isolates. However, one of these isolates (A/Extr/6530/10) carried two unique mutations in the HA head (S88Y and K136N) that were required simultaneously to ablate reactivity with the murine MAbs. Of relevance, this unusual requirement is a direct example of an enhanced antigenic change, a phenomenon postulated to explain the simultaneous fixation of two or more amino acid changes during natural evolution of the influenza H3 subtype and that should be considered when searching for sequence changes in viral isolates that may impact on HA antigenicity. However, the simultaneous changes in A/Extr/6530/10 were not reproduced in escape mutants selected with one of the monoclonals which contained instead single amino acid substitutions in the HA head, near the S88Y and K136N changes. The natural mutations found in A/Extr/6530/10 and the changes selected in escape mutants delineate a new antigenic site in the HA of 2009 pandemic viruses which is rele-

vant for the human antibody response, as shown by competition of MAb binding to virus with human postinfection sera.

Keywords: pandemic influenza, hemagglutinin, antigenicity.

P-108

Cross-immunity against H5N1 in an old-aged vaccinated northern Spanish population

Iván Sanz Muñoz*[1], Raquel Almansa Mora[2], Silvia Rojo Rello[1], José María Eiros Bouza[3], Jesús Francisco Bermejo Martín[2], Raúl Ortiz de Lejarazu Leonardo[1]

(1) Servicio de Microbiología e Inmunología. Hospital Clinico Universitario de Valladolid. (2) Departamento de Investigación Biomédica del Clínico - IBC. Instituto de Estudios de Ciencias de la Salud de Castilla y León . Valladolid (3) Servicio de Microbiología. Hospital Universitario Río Hortega. Valladolid.

Introduction. H5N1 is a highly potential pandemic virus endemic in Shout East Asia, which infects poultry but also can infects human in several cases[1]. Haemagglutinin (HA), a recognition glycoprotein present in surface of flu viruses, is the most antigenic protein in influenza, and the main target for virus-neutralizing Abs[2]. The aim of this study is to check the cross-immunity present in a Spanish population against H5N1 in absence of known circulation of this virus.

Materials and methods. 178 sera from Valladolid National Influenza Center (NIC) were used to check the presence of antibodies that can neutralize Influenza H5N1. Sera came from post-vaccinated people (>65 years) from Castilla y Leon, collected in 2006-2007, 2008-2009, 2009-2010 and 2010-2011 influenza seasons. Antibodies detection was made by means haemagglutination inhibition assays using chicken red cells, and H5 (A/Vietnam/1203/2004) recombinant glycosylated protein (*Protein Sciences®*) was used as antigen. Pre-treatment of sera was made using RDE (*Receptor Destroying Enzyme – Denka Seiken®*) of V. cholerae for digestion of inespecific blood inhibitors accord WHO procedures.

Results. The mean age of sera analyzed was 76 years and 57,3% were males. Six sera out of 178 tested (3, 4%) showed haemagglutination inhibition, two whit titers of 1/320 (two males of 76 and 73 years from 2006-07 and 2009-10 seasons), two whit 1/20 (one male and one woman of 71 and 72 years from 2010-11 season) and two whit 1/10 (one male and one woman of 73 and 80 years from 2009-10 and 2010-11 seasons).

Conclusions. Only few individuals showed haemagglutinin inhibition, and two of them presented protective titers against zoonotic H5N1 subtype (>1/40). Among this repetitive vaccinated population we have demonstrated pre-existing antibodies against H5N1 in less than 5%, and protective behavior in 2%. This phenomenon deserves further explanation to elucidate whether is caused by common epitopes between subtypes or a

broacher inmune response after repetitive flu vaccination in particular individuals.

References. Almansa R, et al. H5 influenza haemagglutinin and cytokine profiles in cultured PBMCs from adults and children. Inmunología. 2011. doi:10.1016/j.inmuno.2011.06.001
Nobuko O, et al. Naturally occurring antibodies in humans can neutralize a variety of Influenza virus strains, including H3, H1, H2 and H5. J. Virol. 2011; 85: 11048-11057

Keywords: influenza A (H5N1), haemagglutinin inhibition.

P-109

Attenuated viruses as a model to study the immunological mechanisms involved in protection against African swine fever

Paula López Monteagudo[1]

(1) Departamento de Infecciones víricas transfronterizas. CReSA. Centre de Recerca en Sanitat Animal. Barcelona.

African Swine Fever (ASF) is a highly infectious viral disease that provokes dramatic losses in the affected countries against which there is no effective treatment neither a vaccine available. Classically, attenuated viruses, despite their biosecurity problems, are an ideal model to unravel the immunological mechanisms involved in protection against ASF. Our immunization model is based on the E75CV1 attenuated virus which was obtained more than three decades ago by Dr. Ruiz-Gonzalvo (INIA-Madrid; Spain), by adapting the ASFV E75L virulent strain to grow in the CV1 established cell line.

Confirming the risks related to their potential use in the field, only the optimal immunization dose behaved as expected for an attenuated virus. Surprisingly enough, both a 10-times higher dose and a 100-times lower dose of E75CV1 proved lethal for some infected animals. Similarly, specific pathogen free (SPF) pigs proved to be much more sensitive to infection with E75CV1 than conventional animals, showing the fine balance between protection and pathogenesis.

As expected, animals immunized with the optimal dose of E75CV1 barely showed visible symptoms of ASF. E75CV1-immunized pigs were able to resist the infection with a lethal dose of the homologous E75L virulent virus and conversely, they couldn't resist the infection with the same lethal dose of BA71, allowing us to classify this virus as a heterologous. New evidences will be presented in our talk showing some *in vitro* correlates with *in vivo* protection.

Independently of the adaptive immune responses induced after E75CV1 infection both the attenuated E75CV1 and the virulent E75L homologous viruses are capable to modulate the host-immune response from very early after infection, albeit they do it in almost opposite ways. Thus, the virulent E75L virus was able to suppress the activation of immune system as early as at 1 day

post-infection (1dpi), allowing the replication and spread of the virus. However, at 7dpi, coinciding with the severe leucopenia and with the death of the animals, a massive activation of pro-inflammatory mediators was detected both by real-time PCR in lymph-node and by ELISA in serum.

Conversely, the attenuated E75CV1 viral strain was immediately recognized by the innate immune system, biasing the adaptive immune response towards a Th1-like response. We really believe that all these results could be very useful for the future rational design of vaccines against ASF.

Keywords: ASFV vaccine, attenuated virus, immunological mechanisms, protection.

P-110

Identification of immunogenic hot spots within rabbit hemorrhagic disease virus (RHDV) capsid protein for efficient antigen presentation

Noelia Moreno[1], Guerra Beatriz[1], Esther Blanco[1], Juan Bárcena*[1]

(1) Centro de Investigación en Sanidad Animal (CISA). INIA. Valdeolmos, Madrid.

Rabbit Hemorrhagic Disease virus (RHDV) is the causative agent of a highly infectious disease of domestic and wild rabbits. RHDV is the prototype strain of the genus Lagovirus within the family Caliciviridae, a group of nonenveloped, icosahedral viruses. Caliciviruses are composed of 180 copies of a single capsid protein.

Our research group has set up a system for the production of large amounts of virus-like particles (VLPs) derived from RHDV, in insect cells (1, 2). Our goal is to develop RHDV VLPs as a delivery system for the multimeric presentation of immunogenic epitopes derived from pathogens relevant for animal health. Currently, we have identified three independent locations within the gene of the RHDV capsid protein (VP60), where we can insert foreign sequences spanning at least 42 aminoacids in length, without affecting the ability of the resulting chimeric protein to self-assemble into VLPs. Moreover, we have demonstrated that the resulting chimeric RHDV VLPs are able to induce strong specific antibody responses against inserted foreign B-cell epitopes, including neutralizing antibodies.

In order to improve the potential of RHDV VLPs as plattforms for foreign epitope presentation, we have performed a PEPSCAN analysis to identificate linear immunodominant regions within the RHDV capsid protein, aimed at defining optimized insertion sites for foreign B-cell epitopes. The results obtained enabled the identification of strong hot spots within the P2 subdomain of the RHDV capsid protein.

References. 1.- Bárcena et al. (2004) *Virology* 322 118-134.
2.- Luque et al. (2012). *Journal of Virology* 86 6470-6480.

Keywords: VLP, PEPSCAN, B-cell epitope.

P-111

Virus-like particles (VLPs) derived from calicivirus as a delivery system for the multimeric presentation of epitopes

Noelia Moreno[1], Esther Blanco*[1], Yolanda Gómez[1], José R. Caston[2], Ignacio Mena[3], Juan Bárcena[1]

(1) Centro de Investigación en Sanidad Animal (CISA). INIA. Valdeolmos, Madrid (2) Departamento de Estructura de Macromoléculas. CNB-CSIC.Cantoblanco, Madrid (3) Department of Microbiology. Mount Sinai School of Medicine. New York, USA

Rabbit Hemorrhagic Disease virus (RHDV) is the causative agent of a highly infectious disease of domestic and wild rabbits. RHDV is the prototype strain of the genus Lagovirus within the family Caliciviridae, a group of nonenveloped, icosahedral viruses. Caliciviruses are composed of 180 copies of a single capsid protein.

Our research group has set up a system for the production of large amounts of virus-like particles (VLPs) derived from RHDV, in insect cells (1, 2). We have identified three independent locations within the gene of the RHDV capsid protein (VP60), where we can insert foreign sequences spanning at least 42 aminoacids in length, without affecting the ability of the resulting chimeric protein to self-assemble into VLPs. Our goal is to develop RHDV VLPs as a delivery system for the multimeric presentation of immunogenic epitopes derived from pathogens relevant for animal health.

We have previously shown the ability of chimeric RHDV VLPs harbouring a foreign cytotoxic T-cell epitope to induce a protective anti-viral response in the absence of adjuvant (3).The aim of the present study was to analyze the potential of chimeric VLPs to induce specific immune responses against foreign B-cell epitopes. To this end we generated chimeric VLPs harbouring B-cell epitopes derived from feline calicivirus or Influenza virus, in different insertion sites. Groups of mice were inoculated with the chimeric VLPs and we analyzed the humoral responses induced. The results obtained indicated that the chimeric RHDV VLPs are able to induce potent antibody responses against foreign B-cell epitopes (including neutralizing antibodies), when inserted at an exposed site within the VLP structure.

In conclusion, RHDV derived-VLPs constitute versatile scaffolds for multimeric antigen display. Moreover, the immunogenic properties of the chimeric RHDV VLPs suggest the potential suitability of these constructions for new vaccine development against animal and human viral infections.

1. Bárcena et al. (2004) *Virology* 322 118-134
2. Luque et al. (2012). *Journal of Virology* 86 6470-6480
2. Crisci et al. (2009) *Virology* 387 303-312

Keywords: VLP, vaccine, antibody response.

P-112

Identification on measles virus hemagglutinin protein of genotype-dependent neutralizing epitopes which are immunogenic both in natural infection and vaccination

Miguel Ángel Muñoz Alía*[1,2], César Santiago[2], José María Casasnovas[2], María Luisa Celma[1], Rafael Fernández Muñoz[1]

(1) Departamento de Virología. Hospital Ramón y Cajal. Madrid (2) Departamento de Estructura de Macromoléculas. Centro Nacional de Biotecnología, CNB-CSIC. Cantoblanco, Madrid.

Background: Measles virus (MV) continue to be a Public Health risk, that currently kills yearly over one hundred thousand children globally, circulating in Asia and Africa and causing outbreaks worldwide. Although MV is serologically monotypic, 24 genotypes have being recognized to date, showing different geographical and temporal distribution. Since our former observation of escape to neutralization by an anti-MV hemagglutinin murine monoclonal antibody (anti-MVH mAb) of virus belonging to one circulating genotype, several studies have suggested that MV circulating strains show antigenic variations, which could potentially affect the efficiency of vaccination.

Objetives: To asses whether genotype-dependent neutralizing epitopes, defined by neutralization escape mutants, are immunogenic in natural infection or vaccination and elicit a long-term B memory.

Materials and methods: We have developed a binding competition radio-immunoassay using a MVH protein in its native oligomeric structure, the major target for neutralizing antibodies. For this purpose we employed (1) MV steady-state persistent infection established in human cells infected with MV primary isolates belonging to different genotypes; (2) human sera from individuals with past infection by MV strains isolated and genotyped years before in our laboratory; (3) I^{125}-labeled anti-MVH mAbs.

Results and conclusions: We have identified and performed a functional and structural characterization of some native epitopes in MVH that selectively bind to IgG present in serum of patients infected years ago by MV belonging to different MV genotypes, indicating that they are immunogenic both in natural infection and vaccination and may elicit a long-term B memory. Thus, it is possible that some MVH epitopes may confer some circulation advantage in partially immunized population depending on the previous exposure to other MV genotypes. These analyses may allow by using serum–libraries and a battery of labeled anti-MVH mAbs to know retrospectively whether in a geographic area at the given time circulated a certain MV genotype and be relevant to design improved measles vaccines and therapeutic anti-MV antibodies.

Keywords: measles virus genotypes; genotype-dependent neutralizing epitopes; human B cell response.

P-113

HPV screening in prevention of cervical cancer: four years results

Elena María Álvarez Alonso[1], Silvia Rojo Rello[1], Iván Sanz Muñoz[1], Lisbeth Gonçalves de Freitas[1], Gabriel March Rosello[1], Sonia Tamames Gómez[2], Raúl Ortiz de Lejarazu Leonardo*[1]

(1) Servicio de Microbiología e Inmunología. Hospital Clínico Universitario de Valladolid. (2) Dirección General de Planificación e Innovación. Consejería de Sanidad de la Junta de Castilla y León. Valladolid.

Introduction. HPV population screening in woman is one of the last added measures for cervix cancer prevention. Castilla y Leon is the unique Spanish community whit a structured cytology program in which since more than four years it was incorporated HPV detection. The aim of this study is to analyze the results obtained at Valladolid.

Materials and methods. It were processed 48.868 endo-cervical samples utilizing *CLART* platform (*Genomica®*), that though a low density microarray system can detect one amplified region by PCR from L1 VPH region, differentiating 35 VPH genotypes whit clinical relevance. It will be compared the results obtained in 2012 whit the results obtained between November 2008 and December 2011.

Results. Since November 2008 till December 2011 it were processed 41.196 endo-cervix samples which 3.297 (8%) were positive. From the positives, it was detected HPV16 in 544 of them (16,5%), HPV18 in 94 (2,8%), HPV6 in 140 (4,2%) and VPH11 in 25 (0,8%). In 2012 it were processed 7.672 endo-cervix samples which 873 (11,3%) were positive, detecting HPV16 in 116 (13,3%), HPV18 in 23 (2,6%), HPV6 in 23 (2,6%) and HPV11 in 9 (1%). It will be commented others results and associations between genotypes.

Conclusions. During last year, it was observed through screening an increase of 37,5 % in detection of HPV. Only HPV16 or in coinfection percentage decreased 20% and HPV18 raised 7%. HPV6 decreased 38% and HPV11 raised 25%. We considered in special interest to continue the surveillance of these four genotypes, because they are included in the vaccine actually used as primary prophylaxis against cervical cancer in Castilla y Leon.

Keywords: HPV, cervical cancer, screening.

P-114

Immune mechanisms involved in the protection elicited by an MVA vaccine against RVFV in mice

Elena López*[1], Gema Lorenzo[1], Belén Borrego[1], Alejandro Brun[1]

(1) Centro de Investigación en Sanidad Animal CISA. INIA. Valdeolmos, Madrid.

This work was aimed to understand the mechanisms involved in the protection elicited by MVA vector vaccines upon a lethal challenge in mice. Since poxviruses are good inducers of type-I and II interferon they have evolved to encode soluble receptors that may counteract their antiviral activity. Due to deletions in the MVA genome, the expression of such antagonists is largely suppressed. We tested the role of type-I interferon responses in the protection of mice immunized with a recombinant MVA expressing the viral glycoproteins (rMVAGnGc). While wild-type 129 mice were protected after viral challenge a significant lack of protection was observed in transgenic 129IFNAR$^{-/-}$ mice. On the other hand MVA is usually a potent inducer of CD8+ T-cell responses. We compared the induction of T-cell responses upon rMVA and DNA vaccination against RVFV, since both vaccine strategies showed good protection levels upon lethal RVFV challenge in Balb/c mice. A collection of class-I MHC predicted 9-mer peptides derived from the glycoprotein (GnGc) and nucleoprotein N primary sequences were tested by an interferon- ELISPOT assay. While none of the N related peptides were able to induce the secretion of significant levels of interferon- , three 9-mer peptides derived from the glycoprotein sequence showed specific stimulation of spleen cells in mice vaccinated with MVA encoding GnGc but not in mice vaccinated with a DNA-GnGc vaccine. Intracellular cytokine staining (ICCS) assays indicated that CD8+ T cells from MVA vaccinated mice were stimulated in the presence of these peptides. Overall these data suggest that successful immunization with the rMVAGnGc vaccine relies in the proper response of the host to type-I interferon and also in the ability to induce detectable levels of specific CD8+ T-cell responses that could contribute to the observed virus clearance in mice challenged with RVFV.

Keywords: RVFV, MVA vaccine, immune response, protection, T-cell epitopes.

P-115

Definition of regulatory genetic elements of a baculovirus expression cassette which significantly improves the production characteristics of the baculovirus vector expression system

Silvia Gómez Sebastián[2], Javier López-Vidal[2], Mª Carmen Nuñez[2], Eva Guijarro[1], José M Escribano*[1]

[1] Departamento de Biotecnología. INIA. Madrid [2] Departamento de R&D. Alternative Gene Expression S.L. UPM. Pozuelo de Alarcón, Madrid .

The baculovirus expression vector system (BEVS) is one of the most powerful, robust and versatile eukaryotic expression system. Baculovirus vectors have proven to have multiple advantages for protein production in a variety of applications because their development speed and versatility for expression of a great variety of protein families. However, the expression yields obtained in insect cells are far away of that obtained with the most productive mammalian cells. In the present work we have defined a baculovirus expression cassette containing

rearranged baculovirus genomic elements that when acting in *cis* and *trans* over specific promoters or combination of promoters, confer to the baculovirus vectors unique properties. One of the elements of this expression cassette is the cDNA *Ac-ie-01* encoding for the transactivators IE1 and IE0. The overexpression of these two transactivation factors induced insect cells proliferation early after infection and conferred to the cells an increased baculovirus resistance, determined by an increase of cell viability at late times post-infection. This increase in cell viability had also consequences in protein conformation, reducing the characteristic proteolysis of recombinant proteins frequently found in the baculovirus-derived proteins. A second set of elements of the expression cassette consisted in a enhancer sequence operatively linked to *p10* promoter or chimeric promoters containing *p10*. These last elements, in combination with the overexpressed IE-1 and IE0, increased the yields of recombinant protein expression from 3 to up to more than 30 times, depending of the time of infection analyzed. Examples of productivities obtained with the baculovirus vectors genetically modified by this expression cassette and expressing different protein vaccines forming virus-like particles will be presented.

Keywords: baculovirus, expression cassette, subunit vaccines.

P-116

A magnesium-dependent RNA structural switch at the Internal ribosome entry site of hepatitis C virus genome monitored by atomic force microscopy

Ana García Sacristán*[1,2], Elena López Camacho[1,3], Ascensión Ariza Mateos[2,4], Miguel Moreno[1], Rosa M. Jáudenes[1,3], Jordi Gómez[2,4], José Ángel Martín Gago[3], Carlos Briones[1,2]

(1) Department of Molecular Evolution. Centro de Astrobiología (CSIC-INTA). Torrejón de Ardoz, Madrid (2) Centro de Investigación Biomédica en Red de enfermedades hepáticas y digestivas (CIBERehd) Spain. (3) ESISNA. Instituto de Ciencia de Materiales de Madrid (CSIC) Madrid. (4) Laboratory of RNA Archaeology. Instituto de Parasitología y Biomedicina "López-Neyra" (CSIC) Armilla, Granada.

Hepatitis C virus (HCV) is the main etiological agent of chronic liver disease in humans. Both 5´and 3´ untranslatable regions (UTR) of the single-stranded RNA HCV genome are highly structured and include regulatory elements necessary for viral replication and translation (1). In particular, the 5´UTR is highly conserved among all HCV genotypes and contains an internal ribosome entry site (IRES) element responsible to drive cap-independent translation initiation (2). The ion-dependent tertiary fold of the minimal HCV IRES element (containing domains II to IV) has been investigated (3), and significant progress has been made in determining the three-dimensional structure of individual

IRES domains and subdomains at high resolution (4). Nevertheless, little information is still available on the tertiary structure of the whole functional HCV IRES element.

Atomic Force Microscopy (AFM) is a useful nanotechnology-based tool for the analysis of a wide range of biological entities, including nucleic acids and their complexes (5). We have optimized AFM technology for analysing HCV IRES structure in native conditions as well as for monitoring its conformational changes in diverse physicochemical environments, in particular at magnesium ion concentrations ranging from 0 to 10 mM. Here we report the magnesium-dependent folding of the HCV IRES in a sequence context that includes its structured, functionally relevant flanking regions (domains I, V and VI). In the 568 nt-long HCV genomic RNA molecule analyzed, a structural switch has been monitored when magnesium concentration increases from 2 to 4 mM. This effect has been confirmed by classical techniques for RNA structural characterization such as gel-shift analysis and partial RNase T1 cleavage. Our results suggest a magnesium-driven transition from an 'open' to a 'closed' conformation of the HCV IRES, at least partially similar to that caused by miR-122 (6). The functional relevance of such an RNA structural switch will be discussed.

1. Lindenbach BD and Rice CM (2005) Nature 436: 933
2. Lukavsky PJ (2009) Virus Res. 139: 166
3. Kieft JS et al. (1999) J. Mol. Biol. 292: 513
4. Berry KE et al. (2011) Structure 19: 1456
5. Hansma HG et al. (2004) Curr. Op. Struct. Biol. 14: 380
6. Diaz-Toledano R et al. (2009) Nucleic Acids Res. 37: 5498

Keywords: Hepatitis C virus (HCV), atomic force microscopy (AFM), RNA structure, Internal ribosome entry site (IRES).

P-117

Characterization of G3BP as a novel IRES trans-acting factor

Alfonso Galán Casan*[(1)], Encarnación Martínez-Salas[(1)]

(1) Departamento de Dinámica y función del genoma. Centro de Biología molecular Severo Ochoa. Madrid.

Internal ribosome entry site (IRES) elements are *cis*-acting RNA regions that were initially characterized in the genomic RNA of picornaviruses. IRES elements recruit the translation machinery with the help of cellular factors termed IRES *trans*-acting factors (ITAFs). Most ITAFs are RNA binding proteins (RBP) that regulate RNA life span and form part of macromolecular complexes that determine the fate of mRNA.

G3BP-1 belongs to a family of RBPs that link tyrosin/kinase receptors-mediated signaling with mRNA metabolism. G3BP-1 contains two RNA binding motifs at the C-termini, a RNA recognition motif (RRM) and an arginine-glycine rich (RGG) motif. G3BP-1 is localized in stress granules (SGs) and contributes to its assembly. SGs are cytoplasmic aggregates containing stalled

pre-initiation complexes, and are thought to serve as sites for mRNA storage during cell stress response (1). During the last years it has been shown that many viruses can modulate SGs assembly to maximize replication efficiency (2).

We previously identified G3BP-1 bound to the foot-and-mouth disease virus (FMDV) IRES through riboproteomic assays (3). Using RNA electrophoretic mobility shift assay and UV-crosslink we have found that purified G3BP-1 interacts directly with specific regions of the FMDV IRES. Mutational analysis confirmed that the preferential binding site is located in domain 5. Functional analysis using *in-vivo* and *in-vitro* translation assays with bicistronic RNAs showed that G3BP-1 acts as a repressor of both cap- and IRES- dependent translation initiation. Furthermore, by using GST-pull down assays we have found that G3BP-1 interacts with polypirimidine tract binding protein (PTB) and translation initiation factor 4B (eIF4B), two host proteins known to stimulate translation initiation.

1. Anderson and Kedersha, 2005. Trends in Biochemical Sciences, 33: 141
2. White and Lloyd, 2012. Trends in Microbiology, 20: 175
3. Pacheco et al, 2008. Proteomics, 8: 4782

Keywords: IRES, FMDV, G3BP-1 and RNA binding proteins.

P-118

Contribution of the 5'UTR of influenza virus mRNAs in the virus gene expresion

Paloma Rodríguez*[1,2], Emilio Yángüez[1,2], Amelia Nieto[1,2]

(1) Department of Molecular and Cellular Biology. Centro Nacional de Biotecnología (CSIC) Madrid (2) Centro de Investigaciones en Red de enfermedades respiratorias (CIBERES).

The influenza virus possesses a single stranded, negative-sense RNA genome composed of 8 different segments. These segments contain 12-13 conserved nucleotides at the 3' and 5' UTRs highly conserved among different RNA segments and virus strains that comprise the viral promoter and signals required for packaging, and polyadenylation. Viral transcription yields the viral mRNA that have a long central coding region flanked by short, untranslated regions or UTRs. Following the 12-13 conserved nucleotides, a 5' UTR sequence of 7 to 33 nucleotide-long in the mRNAs containing segment-specific sequences, has been proposed as required for segment specific regulation.

On the other hand, the viral NS1 protein is a multifunctional protein that performs a plethora of activities, which additionally contributes towards efficient virus replication and virulence during infection. In addition, some of these activities take place through NS1 interaction with influenza non-translated regions genome.

In order to analyze the specific contribution of segment-specific sequences located adjacently to the 12-13 conserved nucleotides and its possible modulation by NS1, we have generated a mutant library at the four adjacent nucleotides in the NS and M segments. Using a combination of transfection/infection system with a RNP reconstitution assay, we have examined the behavior of the different mutants during infection with different viruses, including a recombinant virus lacking NS1. A thorough analysis of these mutants shows how some mutations produce a drastic inhibition of viral polymerase activity, while others seem to increase its activity. In addition, we have identified mutants affected in mRNA transport and/or protein translation. Furthermore, we have found some mutants that seem to be negative regulated by NS1 protein.

Currently, we have rescued viruses that contain the most important mutations and we are analyzing possible differences in viral transcription/replication, viral particle production, interferon response and cytokine production.

The aim of these studies is the characterization of the possible pathogenicity associated to viral sequences that are not constituents of the viral promoter or coding sequences and therefore could represent a virulence associated to natural RNA variation.

Keywords: influenza virus, gene expression regulation.

P-119

RNA aptamers targeting the IRES-binding protein PCBP-2

Miguel Moreno*[1], Eva M. Lanagrán[1], Jorge Ramajo[2], María Fernández-Algar[1], Encarna Martínez-Salas[2], Carlos Briones[1,3]

(1) Department of Molecular Evolution. Centro de Astrobiología (CSIC-INTA) Torrejón de Ardoz, Madrid. (2) Departamento de Dinámica y Función del Genoma. Centro de Biología Molecular "Severo Ochoa" (CSIC/UAM) Madrid (3) Centro de Investigación Biomédica en Red de enfermedades hepáticas y digestivas (CIBERehd), Spain.

Aptamers are single-stranded oligonucleotides (ssDNA or ssRNA) selected from combinatorial libraries by an *in vitro* process. They possess a specific three-dimensional structure depending on their sequence and the physicochemical features of the folding buffer (1). These *in vitro* selected nucleic acids are able to recognize and, eventually, alter the activity of their target molecules by binding to them through non-covalent molecular interactions. The current technology allows generating aptamers with very high affinity and specificity for a broad range of targets including low molecular weight compounds, proteins, nucleic acids and macromolecular complexes. Therefore, aptamer technology has been used in a growing number of diagnostic and therapeutic applications over the last years (2). Aptamers have been adapted to different analytical applications not only as advantageous alternatives to antibodies, but as novel reagents with unique properties.

Several strategies are currently being explored using aptamers against proteins associated with mRNA stabilization, translational activation, and translational silencing (3). Among the complex and diverse set of molecules involved in the regulation of post-transcriptional pathways, the poly(C) binding proteins (PCBPs) are of special interest due to their involvement in internal initiation of translation of some viral RNAs. In fact, initiation of translation of picornavirus RNAs occurs through a cap-independent mechanism that requires the formation of specific ribonucleoprotein complexes involving host cell factors, including PCBP-2 (4,5), and a highly structured RNA functional element of the viral genome located at its 5' untranslated region (5'UTR), known as internal ribosome entry site (IRES) (6). We are selecting and characterizing RNA aptamers against PCBP-2, and our aim is to use them to alter the IRES-mediated translation of foot-and-mouth disease virus (FMDV). Herein, we present preliminary results of the ongoing research in terms of selected sequences, structure analysis and affinity of the aptamers against PCBP-2.

1. Tuerk C and Gold L (1990) Science 249: 505.
2. Cho EJ et al (2009) Annu. Rev. Anal. Chem. 2: 241.
3. Keefe AD et al (2010) Nat. Rev. Drug Discov. 9: 537.
4. Walter BL et al (1999) RNA 5: 1570.
5. Pacheco A et al (2008) Proteomics 8: 4782.
6. Martinez-Salas et al (2008) J. Gen. Virol. 89: 611.

Keywords: aptamers, internal ribosome entry site (IRES), IRES-binding proteins, PCBP-2, foot-and-mouth disease virus (FMDV).

P-120

Development of RNA and DNA aptamers against the IRES-binding protein Ebp1

Eva M. Lanagrán*[1], Miguel Moreno[1], Jorge Ramajo[2], María Fernández-Algar[1], Encarna Martínez-Salas[2], Carlos Briones[1,3]

(1) Department of Molecular Evolution. Centro de Astrobiología (CSIC-INTA).Madrid (2) Departamento de Dinámica y Función del Genoma. Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM) Madrid (3) Centro de Investigación Biomédica en Red de enfermedades hepáticas y digestivas (CIBERehd), Spain.

Aptamers are single-stranded DNA or RNA molecules obtained through a process of amplification-selection termed 'Systematic Evolution of Ligands by EXponential Enrichment' or SELEX (1,2). Over the last two decades, aptamers have gained scientific recognition as therapeutic and diagnostic tools because of their specificity for molecular recognition of a broad range of molecular targets (3). However, a number of issues need to be systematically addressed in order to optimize the *in vitro* processes, including: i) the size and composition of the initial population; ii) the comparison of the performance of DNA aptamers vs. RNA aptamers; and iii) the comparative study of *in vitro* selection versus *in vitro* evolution (4) strategies, and, in the later case, the mutation rate introduced in each enzyme-catalyzed step. Our group is exploring these variables at the sequence/structure levels, through a combined *in vitro-in silico* approach.

Some RNA viruses, including members of the families *Picornaviridae* and *Flaviviridae*, initiate translation of the viral genome following a cap-independent mechanism driven by an internal ribosome entry site (IRES) element (5). Viral IRES elements differ in their nucleotide sequence, RNA secondary/tertiary structure, and the repertoire of trans-acting factors required (5). Among the host cell factors involved in this process, the ErbB3-binding protein 1 (Ebp1), an IRES-trans acting factor also known as ITAF45 and PA2G4, stimulates translation driven by the foot-and-mouth disease virus (FMDV) IRES, but not by other picornavirus IRES elements (6, 7). We have developed DNA and RNA aptamers against Ebp1 through *in vitro* selection (S) and *in vitro* evolution (E) processes in parallel (DNA-S, DNA-E, RNA-S and RNA-E strategies). We are currently studying the nucleic acid folding thermodynamics along the different processes as well as the binding properties (affinity and specificity) of the aptamers against Ebp1. The ability of the most efficient aptamers for interfering with FMDV cap-independent translation initiation will be tested.

1. Ellington AD and Szostak JW (1990) Nature 346: 818
2. Tuerk C and Gold L (1990) Science 249: 505.
3. Klussmann S (2006). The Aptamer Handbook. Wiley-VCH, Germany
4. Joyce GF (2004) Annu. Rev. Biochem. 73: 791.
5. Martínez-Salas E (2008) Trends Microbiol. 16: 230
6. Pilipenko EV et al. (2000) Genes & Dev. 14: 2028
7. Pacheco A et al. (2008) Proteomics 8: 4782

Keywords: picornavirus, foot-and-mouth disease virus (FMDV), internal ribosome entry site (IRES), IRES-binding proteins, Ebp1, aptamers.

P-121

Characterization of viroid subgenomic RNAs: genesis and implications about the mechanisms that regulate viroid titer *in vivo*

Sofia Minoia*[1], Beatriz Navarro[2], Francesco Di Serio[2], Ricardo Flores[1]

[1] Department of Plant Stress Biology. Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC), Universidad Politécnica de Valencia. [2] Department of Plant Virology. Istituto di Virologia Vegetale (CNR) Bari, Italy.

The subgenomic RNAs (sgRNAs), which appear together with those of genomic size (gRNAs) in many ribovirus infections, serve usually for expressing viral proteins. However, the functional role that sgRNAs may play in viroids is a conundrum, because these infectious are small RNAs with autonomous replication but without protein coding capacity. In the course of our studies on *Potato spindle tuber viroid* (PSTVd), the type species of the family *Pospiviroidae,* we have consistently observed a pattern of 6-7 sgRNAs that accompany its (nuclear) replication in an experimental host (eggplant). The isolation and analysis by primer extension of these sgRNAs has revealed that they do not seem to be products of the premature termination of transcription, since they lack the common 5′ termini to be expected if strand elongation were to start from an specific position (as in the family *Avsunviroidae).* Further examination by RLM-RACE *(RNA ligase-mediated*

rapid amplification of cDNA ends), besides confirming the 5′ termini of the sgRNAs, has shown that they contain a free hydroxyl group because RLM-RACE is dependent on a previous phosphorylation. This observation suggests that the sgRNAs result from cleavage of the gRNAs, either during their replication or once it is completed, catalyzed by typical RNases that generate this class of termini and act preferentially on unpaired regions. The alternative possibility, involving other RNases like those of class III and RISC characteristic of RNA-mediated gene silencing, is unlikely because they generate 5′ phophomonoester termini. However, the linear gRNAs (or at least part of them) show this terminal group, in consonance with our previous results (Gas et al., *J. Virol.* 2007) implicating one or more RNases of class III in the cleavage of the oligomeric RNAs (generated by a rolling-circle mechanism) to give rise to the unit-length linear forms. We are presently analyzing the 3′ termini of the sgRNAs, to confirm and define better the pathway underlying their genesis. This pathway, together with replication, determines presumably the final accumulation levels of viroid gRNAs *in vivo.*

Keywords: viroids, non-protein-coding RNAs, subgenomic RNAs.

P-122

Interferon regulates the expression of several non-coding RNAs

Elena Carnero[1], Marina Barriocanal[1], Victor Segura[2], Puri Fortes*[1]

(1) Department of Gene Therapy and Hepatology. CIMA. Pamplona (2) Department of Bioinformatics. CIMA. Pamplona.

Transcriptome analysis has recently shown that cells express a high number of long non-coding RNAs (lncRNAs) of unknown function. It has been determined that the expression of some lncRNAs is altered in response to disease. In fact, some play important roles in cell proliferation and differentiation. However, few studies have analyzed their role in cell homeostasis or in cell response to extracellular stimuli. Interferon (IFN) a in a potent cellular stimuli against viral infection and has a crucial role in the treatment of HCV chronic infection. Many protein coding genes have been described to mediate IFN response, however the relevance of non-coding RNAs in the response to IFN is unknown. To determine whether lncRNAs could mediate IFN functionality, we have first analyzed the transcriptome of Huh7.5 controls or IFN-treated cells by Sure Print microarray and RNASeq. Analysis of the microarray showed altered expression with high statistical significance of 845 probes, 90% of which were up-regulated and represent the well-characterized transcription activation pattern of IFN. Similar results were obtained after analysis of RNASeq. We have analyzed the genes annotated as lncRNAs in the

array. In contrast to coding genes, 40% of the probes were down-regulated, suggesting an unexpected role of the IFN response in transcription inhibition. We have validated 12 of the 18 lncRNAs up-regulated annotated in Ensembl. The results show different kinetics of activation ranging from 2 to more than 100 fold. Most of these lncRNAs respond to IFN in all cell-lines tested and accumulate preferentially in the nucleus. However, some of them are mostly in the cytoplasm or move to the cytoplasm after IFN treatment. Most respond to IFN alpha, beta and lambda, but interestingly one seems to be more sensitive to IFN lambda, a type III IFN. Thus, differences in lncRNA expression could result in the different strength and kinetics of response observed for type I or type III IFNs. Finally we also determined whether some of the lncRNAs upregulated by IFN are also induced after HCV infection. The results show that eight out of eighteen lncRNAs were also upregulated in cells infected with HCV. Further studies will be required to understand the role of these lncRNAs both in IFN response and HCV infection.

Keywords: LncRNAs, HCV, IFN, antiviral.

P-123

Expression of artificial microRNAs: an antiviral strategy in plant biotechnology

Frida Mesel Casanova*[1], Mingmin Zhao[1], Beatriz G. García[1], Juan Antonio García[1], Carmen Simón Mateo[1]

(1) Departamento de Genética Molecular de Plantas. Centro Nacional de Biotecnología CSIC . Cantoblanco, Madrid.

MiRNAs are important regulators of gene expression in both plants and animals. miRNAs are single-stranded RNAs, 20–24 nucleotides in length, generated from processing of longer pre-miRNA precursors. These miRNAs are recruited to the RISC complex and in a sequence-specific manner can down regulate target mRNAs in several ways. Plant viruses cause important losses to modern agriculture. *Plum pox virus* (PPV) is a persistent threat to the production of stone fruit trees of the *Prunus* genus, and novel approaches for protection are needed. Trying to explore new strategies to develop virus resistance, we have developed artificial miRNAs (amiRNAs) against PPV. Based on previous RACE analysis of RISC-cleavage ends and on deep sequencing analysis, some amiRNAs were designed and assembled into a pre-miRNA backbone derived from the natural *Arabidopsis thaliana* pre-miR159. Moreover, a dimer amiRNA has been obtained to produce in the same construct two amiRNAs against PPV genome. Single and dimer amiRNAs have been agroinfiltrated into *Nicotiana benthamiana* to as-

sess by Northern-blot analysis amiRNA accumulation of both mature and star amiRNAs strands and, unexpectedly, in most of cases the accumulation of star strand was higher than that of mature strand. Strand asymmetry appeared not to depend on bulges in the stem region of the pre-amiRNA hairpin. The efficiency of protection against PPV has been assessed in a bioassay using transient expression in *N. benthamiana* of the different amiRNA constructs and the protection against the virus occurred using either the individual and dimer amiRNAs. Indeed, the most active amiRNAs against PPV were selected to be expressed in *N. benthamiana* transgenic plants. Most of the transgenic lines showed resistance to the PPV challenge. In some transgenic plants, virus variants with different mutations in the amiRNA targets were selected. The antiviral strategy explored in this work has clear biotechnological application and could be also applied to other plant pathogens. However, extensive research is still required to understand the factors affecting the broadness and durability of the resistance achieved with this experimental approach.

Keywords: plant virus resistance, artificial miRNA, PPV-resistant transgenic plants.

P-124

Profile of Pelargonium line pattern virus-derived small RNAs from *Nicotiana benthamiana* plants

Marta Blanco Pérez*[1], Miryam Pérez Cañamás[1], Javier Forment Millet[2], Carmen Hernández Fort[1]

[1] Departamento de Virología Molecular y Evolutiva de Plantas. Instituto de Biología Molecular y Celular de Plantas Primo Yufera. Valencia [2] Departamento de Bioinformática. Instituto de Biología Molecular y Celular de Plantas Primo Yufera. Valencia.

In plants, RNA silencing functions as an antiviral mechanism that is triggered by double-stranded or highly structured single-stranded RNA which serve as a substrate for Dicer-like ribonucleases (DCL) to produce virus small RNAs (vsRNAs) of 21-24 nt. These small duplexes are unwound, and one strand is incorporated into Argonaute (AGO)-containing effector complexes that can then target complementary viral RNA for degradation. *Pelargonium line pattern virus* (PLPV) has a monopartite positive sense RNA genome of 3883 nt that contains five ORFs encoding two replicases (p27 and p87), two movement proteins (p7 and p9.7) and a protein (p37) with a dual role in encapsidation and in suppression of RNA silencing. Though PLPV shows resemblances with carmoviruses at genomic and protein level, it exhibits dissimilar expression strategies, particularly because it produces not two, but one single subgenomic RNA for expression of the internal and

3´-proximal ORFs. This has prompted the proposal of its inclusion into a prospective new genus (*Pelarpovirus*) in the family *Tombusviridae* (Castaño *et al*., 2009, *Virology* 386:417-426). As commonly observed in its natural hosts (*Pelargonium* spp.), PLPV establishes systemic, low-titered and asymptomatic infections in a variety of experimental hosts, including *Nicotiana benthamiana*. As in other plant-virus interactions, the particularities of the PLPV infection process may be largely determined by the silencing-based host defence and the corresponding viral counter-defence, with the vsRNAs being a main component of the interplay between the host and the pathogen. Here we show that PLPV infection in *N. benthamiana* occurs with the accumulation of high levels of vsRNAs and, throughout deep-sequencing, blot hybridization and computational approaches, we have obtained information on their molecular properties and distribution along the viral genome. The data indicate that PLPV sRNAs: i) represent more than 90 % of total sRNAs in infected tissue, ii) are predominantly of 21-22 nt, with those of minus polarity being in moderate excess with respect to those of plus polarity, iii) display a bias in their 5´-terminal nucleotide and, iv) derive from the entire virus genome though there is a prevalent contribution of certain regions. In the light of these results, the nature of the viral substrates that trigger silencing as well as the potential identity of the DCL and AGO proteins involved in anti-PLPV defence are discussed.

Keywords: vsRNAs, plant viruses, perlargonium line pattern virus, RNA silencing.

P-125

Dahlia latent viroid, a recombinant new species of the family *Pospiviroidae*: the question of its origin and classification

Jacobus Th. J. Verhoeven[2], Ellis T. M. Meekes[2,3], Johanna W Roenhorst[2], Ricardo Flores[1], Pedro Serra*[1]

(1) Department of Plant Stress Biology. Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC), Universidad Politécnica de Valencia. (2) National Reference Centre. National Plant Protection Organization. Wageningen, The Netherlands (3) Department of Plant Protection Naktuinbouw. Roelofarensdsveen. The Netherlands.

Examination by return and double polyacrylamide gel electrophoresis of two asymptomatic dahlia accessions disclosed the presence of a viroid-like RNA that appeared smaller than *Chrysanthemum stunt viroid* and *Potato spindle tuber viroid*, the two members of the genus *Pospiviroid*, family *Pospiviroidae,* reported in this ornamental plant previously.RT-PCR with primers designed for amplifying all pospiviroids produced no amplicons, but RT-PCR with random primers revealed a 342-nt RNA. The sequence of this RNA was confirmed by RT-PCR with specific primers, which additionally revealed its wide natural distribution: five out of ten samples from pot-grown dahlia and 92 (belonging to 71 cultivars) out of 100 samples from field-grown dahlia were infected. The RNA was named *Dahlia latent viroid* (DLVd) because it repli-

cates autonomously, but without inciting symptoms, in dahlia and shares maximum sequence identity with other viroids of less than 56%. Furthermore, DLVd displays characteristic features of the family *Pospiviroidae:* a predicted rod-like secondary structure of minimum free-energy with a central conserved region (CCR), and the ability to form the metastable structures hairpin I and II with which key functional roles have been associated. Its CCR is identical to that of *Hop stunt viroid* (HSVd, genus *Hostuviroid*). However, DLVd: i) has the terminal conserved region present in members of the genus *Pospiviroid,* but absent in HSVd and, ii) lacks the terminal conserved hairpin present in HSVd. Phylogenetic reconstructions indicate that HSVd and *Pepper chat fruit viroid* (genus *Pospiviroid)* are the closest relatives of DLVd,but DLVd differs from these viroids in its host range, restricted to dahlia so far. Therefore, while DLVd fulfills the criteria to be considered a new species of the family *Pospiviroidae,* its recombinant origin makes assignment to the genera *Pospiviroid* or *Hostuviroid* problematic and leaves open the alternative of creating a new genus.

Keywords: viroids, non-protein-coding RNAs, ornamentals.

P-126

Usefulness of a real-time quantitative PCR for the diagnosis of congenital and postnatal cytomegalovirus infection

Jordi Reina*[1], Irene Weber[1], María Busquets[1], Carmen Morales[1]

[1] Virology Unit. University Hospital Son Espases. Palma de Mallorca.

Introduction: Cytomegalovirus (CMV) is the leading cause of virus infections and postnatal defects in children.

Objective: The objective of this study is to evaluate the usefulness of a real-time quantitative PCR against a qualitative PCR and shell-vial cell culture in the diagnosis of these infections using urine as a single sample.

Patients and Methods: We studied all the urine samples of newborns (<7 days) with suspected congenital infection and urine of patients with suspected postnatal infection (urine negative birth). Urines were simultaneously studied by cell culture-type shell vial (line MRC-5, Vircell), qualitative PCR (PCRcl; Real Durviz, Granada) and quantitative real-time PCR (PCRq; Abbott RealTime CMV, Germany).

Results: We analyzed 332 urine samples (270 to rule out congenital infection and 62 postnatal infection). Of the first 22 (8.1%) were positive in the PCRq, 19 (7.1%) in the PCRcl and 17 (6.2%) in culture. By comparing the culture with the rest of the PCRq techniques had a sensitivity of 100%. Using the PCRq as a reference technique, the cul-

ture had a sensitivity of 77.2% and PCRcl 86.3%. The PCRcl values when compared to the culture have been of a 100% sensitivity and 99.2% specificity. In cases of infection postnatal PCRq detected 16 (25.8%) positive cases, the PCRcl 12 (19.3%) and cell culture 10 (16.1%). The urine presented viral loads ranging between 2,178 and 116,641 copies/ml. In both types of studies no urine was positive and negative in the PCRs. CMV viral load in urine culture negative initially ranged between 1,200 and 5,430 copies/ml. The viral load in the urine with PCRq positive and PCRcl negative was always less than 5,000 copies/ml.

Conclusions: The PCRq real-time genomic amplification technique has been more sensitive than the other techniques discussed. This technique should be considered as reference (gold standard) leaving the cell culture as second line. The low cost and PCRq automatization would enable the screening of CMV infection in neonatal and post-natal large populations through the use of pools of urine.

Keywords: Cytomegalovirus; Congenital infection;Molecular diagnosis.

P-127

Preliminary evaluation of an immunochromatographic (IC) method for rapid and simultaneous detection of influenza virus A, influenza virus B, RSV and adenovirus in pediatric respiratory samples

Jordi Reina*[1], Irene Weber[1] María Busquets[1], Carmen Morales[1]

[1] Virology Unit. University Hospital Son Espases. Palma de Mallorca.

Aims: To evaluate the usefulness of a new IC system for rapid, specific and simultaneous antigenic detection of the principal viruses causing acute respiratory infections in pediatric patients.

Material and Methods: For this study we have used samples (nasopharyngeal aspirates) from pediatric patients (<10 years) previously positive to each of the viruses studied and stored at -20ºC. For the evaluation of RSV we used 30 positive samples by cell culture (Hep-2, Vircell, Granada) and by IC routine detection (Directigen EZ RSV, Bacton & Dickinson, USA). For respiratory Adenovirus 10 positive samples were used; all were positive on culture (Hep-2, Vircell) and antigen detection by immunofluorescence (MonoFluokit Adenovirus, BioRad, Ireland). For influenza B (influenza B) 10 samples were used positive on cell culture (LLC-MK2, Vircell) and routine IC antigenic detection (Directigen EZ-FluA + B). For influenza A (influenza A) subtype (H3N2) were used 15 samples positive by culture (MDCK, Vircell) and IC, and for influenza A (H1N1) pdm09 15 samples positive by cell culture and IC. The eval-

uated IC detection system is called CerTest Influenza A/B+RSV-Adeno Resp. (Combo Card Test) (Zaragoza, Spain). For the comparative study we carefully followed the manufacturer's recommendations. All results were interpreted by a single observer without knowledge to the previous results.

Results: In the detection of RSV, the CerTest detected 20 positive samples (sensitivity 66%). In the respiratory Adenovirus detection, the CerTest detected 2 positive samples (20% sensitivity). In the detection of influenza virus B the CerTest detected 3 positive samples (30% sensitivity). In the detection of influenza virus A (H3N2) the CerTest detected 10 positive samples (sensitivity 66%) and in the detection of influenza virus A (H1N1) pdm09 CerTest the detected one positive sample (6% sensitivity).

Conclusions: The IC rapid antigen detection system CerTest revealed a low sensitivity especially from detection of respiratory adenovirus, influenza B and influenza A (H1N1) pdm09. 10 minutes of reaction for the test appear insufficient for the detection of weak positivity of samples. The new test should be improved for routine use.

Keywords: antigenic detection, immunochromatography, respiratory viruses.

P-128

Prospective study of Influenza C in hospitalized children

Belén Borrell Martínez*[1], Cristina Calvo Rey[1], Mª Luz García García[1], Francisco Pozo[2], Inmaculada Casas[2]

(1) Department of Pediatrics. Severo Ochoa Hospital. Leganés, Madrid (2) Influenza and Respiratory Viruses Laboratory. National Center of Microbiology, Instituto de Salud Carlos III. Madrid.

A prospective study was conducted to determine the incidence and clinical manifestations associated with influenza C infection in hospitalized children in Spain.

Patients and methods: Between September 2005 and July 2012, all children under 14 years of age, who were admitted at the secondary public Severo Ochoa Hospital (Leganés, Madrid) with respiratory tract infection, were enrolled.

During the hospital stay a phisician filled out a study-questionnaire with the clinical variables. Specimens of nasopharyngeal aspirates (NPA) were sent for virological study at the Respiratory Virus Laboratory, in the National Centre of Microbiology (ISCIII, Madrid, Spain).

Three RT-nested PCR assays were performed to detect by using a previously described method including only the primer sets to amplify influenza viruses in a multiplex PCR assay.

A second multiplex PCR was used to detect parainfluenza viruses 1 to 4, human coronaviruses 229E and OC43, enteroviruses and rhinoviruses.

Presence of RSV-A and B types, HMPV, HBov and adenoviruses were investigated by a third multiplex RT-nested PCR method.

Results: A total of 2,687 hospitalized children were enrolled and 76.4% of analyzed specimens showed positive for some viral agent. Influenza infection was documented on 128 samples (6.2%).

Influenza A was 60% and influenza B, 16.4%. Seventeen patients with confirmed influenza C infection were identified (13.3% of influenza positive cases).

Only 4 patients had a simple infection of influenza C, meanwhile the 81% of the cases had a multiple infection with one, two or three viruses. The patients with simple influenza C infection were clinically similar to other cases. The diagnosis were recurrent wheezing (2 had infiltrate in the chest x-ray) and pneumonia without wheezing. All of them had fever.

Conclusions: Influenza C infections accounted for 13,3% of influenza positive cases. The patients with simple influenza C infection were clinically similar to other influenza types, but a high rate of co-infection makes it difficult to assess its clinical role.

1. Coirad MT et al. Simultaneous detection of fourteen respiratory viruses in clinical specimen by two multiplex reverse transcription nested-PCR assays. J Med Virol 2004;72:484-95
2. Calvo C et al. Detection of new respiratory viruses in hospitalized infants with bronchiolitis: a three-year prospective study. Acta Pediatrica 2010; 99:883-7

Keywords: influenza C, hospitalized children, respiratory infections, bronchiolitis, wheezing.

P-129

Effect of sample analysis delay in the results of enteric virus detection in food

Marta Díez Valcarce*[1], Marta Hernández Pérez[2], Nigel Cook[3], David Rodríguez Lázaro[2,4]

[1] Departamento de Biotecnología y Ciencia de los Alimentos. Universidad de Burgos. [2] Laboratorio de Biología Molecular. Instituto Tecnológico Agrario de Castilla y León. Valladolid [3] Department of Food and Environmental Microbiology. Food and Environment Research Agency. York, UK [4] Área de Microbiología. Universidad de Burgos.

Food samples are not always analysed immediately after its collection. The inclusion of a Sample Process Control Virus (–SPCV-), a non-target virus added to each sample at the beginning of the process, is an essential tool for the assessment of the entire process from the extraction step to the molecular detection of the pathogen as it guarantees the accurate recognition of a failed or incorrect performance of the pre-amplification steps in the analysis of food samples.

In this study, we inoculated two food matrices (strawberry and iceberg lettuce) frequently associated with viral foodborne outbreaks with known quantities of two SPCVs (murine norovirus -MNV-1- and a genetically-modified mengovirus -vMC_0). Both viruses were added at three different stages of the concentration-extraction protocol (on the surface of the foodstuff before starting the procedure, in the buffer used for viral elution and in the

supernatant recovered after the first centrifugation of the sample). Besides, we intentionally elapsed the time until the beginning of the analytical procedure after the addition of the SPCVs at five different times: immediately after the SPCV has dried up from the food surface-time 0-, and after 1, 2, 4 and 24 hours. We calculated the efficiency of the extraction. Statistical analyses (ANOVA or non-parametric tests) were performed and no significant differences were observed regarding the time elapsed from the addition of SPCV. However, significant differences were observed depending on the step in which the SPCV was incorporated: the extraction efficiencies were higher if the SPCV was incorporated later, which demonstrate that virus lost occurs along the process.

Consequently, the addition of the SPCV at the beginning of the analysis is recommended to allow a correct monitoring of the procedure. The results also demonstrate that the commencement of the sample analysis procedure after the SPCV addition can be postponed, at least, up to 24 hours without any significant effect in the outcome of the analysis.

Keywords: sample process control virus, eealtime PCR, false negatives, enteric viruses, food.

P-130

Epidemiology and clinical association of parechoviruses: understanding a new infection in children

María Cabrerizo*[1], Cristina Calvo[2], Gloria Trallero[1], David Tarragó[1], Francisco Pozo[1], María Luz García García[2], Inmaculada Casas[1]

(1) Department of Virology, National Centre for Microbiology (NCM). Instituto de Salud Carlos III. Majadahonda, Madrid (2) Department of Pediatrics. Hospital Severo Ochoa. Leganés, Madrid.

Introduction. Human parechoviruses (HPeVs) are RNA viruses of the *Picornaviridae* family. Types 1 and 2, originally described as echoviruses 22 and 23 within Enterovirus genus, were isolated over 50 years ago, whereas further 14 HPeV types have been recently identified. Infections are prevalent in young children and have been associated with mild respiratory and gastrointestinal diseases, as well as with meningitis, encephalitis and neonatal sepsis. In Spain, there are no studies about their epidemiological and clinical associations, in part because HPeVs are not yet included in the routine viral diagnosis of those diseases.

Objective and Methods. To investigate the involvement of HPeVs in pediatric respiratory and neurological infections, two retrospective studies were carried out with clinical samples from children diagnosed as acute respiratory infection (ARI), meningo-encephalitis, febrile syndrome or neonatal sepsis. 504 nasopharyngeal aspirates

(NPAs) and 307 sera or cerebrospinal fluids (CSFs) were studied. Samples were received during 1 yr in the NCM for virological diagnosis. In NPAs, the respiratory viruses included in the routine diagnosis are FLU, AdV, RSV, HRV, HBoV and PIV. All sera/CSFs resulted negative for herpesviruses (HSV-1, HSV-2, VZV, HHV-6) and enteroviruses. For HPeV detection, a real-time RT-PCR in the 5'-untranslated region of the genome was designed. Genotyping was performed using a RT-PCR in the VP3/VP1 region and posterior sequencing and phylogenetic analysis.

Results and Conclusions. The results of our study in Spain confirm others previously reported. Epidemiology of HPeV infections depends on the detected type. HPeV-1 and -6 are predominant in ARI, affecting children between 3 months and 5 years, while HPeV-3 causes mainly neurological manifestations and sepsis in children up 7 months of age. Each HPeV type shows a specific seasonal distribution. HPeV frequency in ARI is relatively low (3.7% in our series) with a high number of co-detections (89%) and, as occurs with other respiratory viruses, there is no a clear association between the detection of HPeVs and the clinical pathology. However, in meningo-encephalitis and neonatal sepsis, the significant HPeV-3 incidence (7.8%) and the short age of the patients (71% of HPeV-positive samples were from neonates) confirm the important role of these viruses in the mentioned pathologies to considerate seriously the inclusion of their detection in the routine laboratory diagnosis.

Keywords: pediatric infections, HPeV, neonatal sepsis, RT-PCR, routine diagnosis.

P-131

Evaluation of a new chemiluminiscent immunoassay for the detection of HSV-1 IgG

Eulalia Guisasola[(1)], Jesús de la Fuente[(1)], Fernando de Ory*[(1)]

(1) Departamento de Virología. Centro Nacional de Microbiología. Majadahonda, Madrid.

There are two herpes simplex viruses (HSV), HSV-1 and -2, showing a high degree of crossreactivity. The use of their respective glycoprotein G, gG1 and gG2, ensures the characterization of type specific serological response. There are different serological approaches for HSV serology, mainly enzyme immunoassay (ELISA), immunochromatography and immunoblot. Recently, a new immunoassay for HSV-1 IgG based on a chemiluminiscent immunoassay (BIO-FLASH^{R} HSV-1 IgG, Biokit, Spain), has been developed. The aim of this study was to compare the performance characteristics of this new assay, as done on the fully automated BIO-FLASH^{R} Instrument (Biokit), using as reference an indirect ELISA (HerpeSelect 1 ELISA IgG, Focus Diagnostics, California, USA). Both assays use recombinant gG1 as antigen. Serum specimens (n=438) received for routine HSV serology in a clinical laboratory were included in the study. The assays in comparison were done strictly following manufacturer´s instructions. For final classification samples showing discrepant results were retested in duplicate by both assays. When assayed by HerpeSelect 1 ELISA IgG, 314 samples were positive, 114 were negative, and the remaining 10 samples showed equivocal result (3 positive and 7

positive when tested by BIO-FLASH^{R} HSV-1 IgG). Equivocal samples were excluded from the calculations. Agreement in both methods was 99.07% (424/428). The sensitivity of BIO-FLASH^{R} HSV-1 IgG was 99.36% since positive result was obtained in 312 out of 314 ELISA positive samples. On the other hand, negative result was obtained in 112 of 114 ELISA negative samples tested; the specificity being 98.25%. The evaluated fully automated BIO-FLASH^{R} HSV-1 IgG system provides comparable results to those of ELISA, reducing hands-on time and eliminating the necessity to aliquot specimens prior to testing. An additional advantage of BIO-FLASH^{R} HSV-1 IgG was the absence of equivocal results allowing the clear classification of samples.

Keywords: herpes simplex virus, HSV-1, chemiluminiscent immunoassay, ELISA.

P-132

Clinical validation of a chemiluminiscent immunoassay for measles IgG and IgM

Fernando de Ory*[1], Teodora Minguito[1], Pilar Balfagón[1], Juan Carlos Sanz[2]

(1) Dpto.de Virología. Centro Nacional de Microbiología. Madrid (2) Dpto. de Microbiología Clínica. Laboratorio Regional de Salud Pública. Madrid.

In the context of the measles elimination plan, the identification of recent measles infections is an important issue for clinical laboratories. Diagnosis of measles is made by PCR and serology, by detection of IgG and IgM. Recently a fully automated chemiluminiscent immunoassay (CLIA) (Liaison, Diasorin, Italy), using a recombinant antigen has been applied for measles antibody detection. The aim of this study was to evaluate this new assay.

Samples were selected on the basis of the results obtained by indirect ELISA (Enzygnost, Siemens, Germany). For measles IgM, 204 serum samples were analyzed. Of them, 50 were IgM positive, 104 were IgM negative, showing specific IgG, and 50 samples came from recent infection due to other viruses (B19V, rubella, mumps, CMV and EBV, 10 of each). For measles IgG assay, 162 samples were tested: 106 and 56 were, respectively, IgG positive negative in ELISA. All the samples were tested by CLIA and ELISA. Discrepant results were retested, and assayed by indirect immunofluorescence (IIF) (EuroImmun, Germany); the final result was the one obtained in this assay.

For measles IgM, when IgM ELISA positive samples were tested by CLIA, positive result was obtained in 47, indeterminate in 1, and negative in 2. Thus, the sensitivity on CLIA against ELISA was 94%. All the three discordant samples were negative in IIF; thus, the corrected sensitivity was 100%. On the other hand, all the measles IgG positive/IgM negative samples, and the samples from other viral infections showed negative result, with a specificity value of 100%. When IgG ELISA positive samples were tested by CLIA, positive result was obtained in 103, indeterminate in 1, and negative in 2. When analyzing ELISA IgG negative samples, 52, 2 and 2 were positive, in-

determinate and negative, respectively. Excluding from the calculations the samples with indeterminate results, the sensitivity and specificity of CLIA against ELISA were respectively 98.1% and 96.3%. After IIF testing of discrepant samples, 110 were finally classified as positive and 52 as negative. Amongst positive samples 105 were positive, 3 indeterminate and 2 negative in CLIA; excluding indeterminate samples, sensitivity was 98.13%. All negative samples showed negative result (specificity 100%). As conclusions, the fully automated Liaison showed excellent sensitivity and specificity values in its application to measles IgG and IgM, eliminating the need for aliquoting specimens prior to assay.

Keywords: chemiluminiscent immunoassay, ELISA, measles IgM.

P-133

Unique european oseltamivir resistant influenza A(H3N2) virus in a immunocompromised patient detected in 2012 in Galicia

Inmaculada Casas*[1], María Teresa Cuevas[1], Isabel López Miragaya[2], Sonia Peres[2], María del Carmen Albo[3], Mónica González Esguevillas[1], Ana Calderón[1], Mar Molinero[1], Silvia Moreno[1], Unai Pérez[1], Francisco Pozo[1]

[1] Influenza and Respiratory Virus Unit. National Influenza Center, CNM, ISCIII. Majadahonda, Madrid [2] Microbiology Department. Complexo Hospitalario Universitario de Vigo. [3] Hematology Department. Complexo Hospitalario Universitario de Vigo.

Introduction. Influenza in immunocompromised patients is often associated with prolonged viral shedding. The resistant oseltamivir viruses in treated patients presented the E119V/I, R292K and N294S mutations in the NA gene

Objectives. We study the unique, as far as we know, European oseltamivir resistant influenza A(H3N2) virus isolated from a treated immunocompromised patient with prolonged shedding of sensible, mixed population and resistant viruses

Case report. In Jan 2011, the patient was diagnosed with precursor T acute lymphoblastic leukemia. After 5 months, underwent umbilical cord blood for allogeneic transplantation

-On Jan 11th 2012, respiratory symptoms appeared and false negative result for influenza A virus was recorded in NPA (Sample A)

-On Feb 5th, respiratory difficulty persisted. A second NPA (Sample B) was studied resulting positive for A(H3N2) which was isolated in MDCK cell cultures

-On Feb 11th, the first treatment was established with oseltamivir 75mg/12h/7d. No X-ray alterations were observed until that date

-On Feb 13th the patient was admitted to hospital referring vomiting, diarrhea and a general deterioration. The tested BAL (Sample C) and a nasal lavage (Sample D) resulted positive. Isolation was also pos-

itive from both samples. X-ray showed interstitial bilateral infiltration viral origin

In **s**amples taken before oseltamivir treatment, the sequences of the NA gene revealed an E119 aa as the vaccine virus 100% sensible to oseltamivir (Samples A, B). After the 1st antiviral treatment (Samples C and D) sequences and pyrosequencing of the NA revealed a mixed population of viruses (87% E119V, 13% E119)

-On 7th Mar, the patient received the 2nd treatment with oseltamivir 75mg/12h/14d and was discharged from hospital with the infection apparently resolved on 27th March

-On 18th Apr, fever, cough and vomits reappeared. A NPA was taken (Sample E) and was negative

-On 27th Apr a new NPA was taken (Sample F) without respiratory symptoms and resulted positive for A(H3N2) which was isolated

-On 9th May, a new NPA (Sample G) was also positive indicating that the influenza A infection remained unresolved

After the 2nd treatment (samples F and G) the sequences and pyrosequencing of the NA revealed the acquisition of resistance to oseltamivir due to the E119V substitution 100%

-During May multiple complications appeared with acute abdomen and acute cholecystitis, respiratory insufficiency and cough. The patient died 1st June

Keywords: Influenza A(H3N2), oseltamivir, immunocompromised.

P-134

Detection of CMV in dried blood spots. Sensitivity of a technique based on genome amplification

Óscar Martínez[1], José A Boga[1], Marta E. Álvarez Arguelles[1], Antonia Templado[1], Carmen Rodríguez Ledo[1], María de Oña*[1], Santiago Melón[1]

[1] Servicio de Microbiología. Hospital Universitario Central de Asturias. Oviedo.

Introduction: Cytomegalovirus (CMV) is the most common cause of congenital infection in developed countries. More than 60% of children, which develope sequels (psychomotor retardation and sensorineural deafness), were asymptomatic at birth. In these cases, the only method to differentiate congenital and perinatal infection is the detection of CMV in dried blood spots (DBS), wich is collected on filter paper during the first week of life for neonatal screening metabolopathies.

Objectives: To determine the sensitivity of a real-time PCR to detect CMV in DBS. To check the validity of this technique for the diagnosis of congenital CMV infection outside the neonatal period and to monitor viral load in children with congenital infection.

Material and methods: 61 whole bloods from patients with detectable CMV by an "in house" real time- PCR (RT-PCR)were selected (average cycle threshold (Ct) value for all samples: 35.03 ± 2.92, range (27.39-39). 25 ul of whole blood is deposited on filter paper (Whatman 903, What-

man Ltd.). The blood is eluted from the filter paper by adding 100 µl of EMEM and incubation at 56°C for 30 minutes. CMV genome, previously extracted, is detected by a RT-PCR. The parameter Ct was determined.

Quantificatión: Ct 27-30 ($5x10^5$ DNA copies/ml); Ct 30-33 ($5x10^4$ DNA copies/ml); Ct 33-36 ($5x10^3$ DNA copies/ml), Ct >36 (< 500 DNA copies/ml).

Results: CMV genome was detected in 28 (45.90%) samples with an average Ct value of 38.24 ± 2.06, range (27.39-37). Samples were grouped depending on the Ct values obtained in the quantification of the whole blood.

	Ct			p
	27-33	33-36	>36	
n	17	17	27	
average (whole blood)	31.10±1.55	34.85±0.79	37.63±0.87	
average (DBS)	37.79±1.83	38.68±1.93	40.37±2.37	
sensitivity	82.35%	58.82%	14.81%	0.016

In those samples whose Ct value range was 27-33, no significant differences appear between the sensitivity of the RT-PCR in DBS and that in whole blood. Significant differences appear between the sensitivity of the RT-PCR in DBS in those samples whose Ct value range was 27-33 and that in those samples with higher Ct value ranges.

Conclusions: 1) The sensitivity of CMV detection by RT-PCR in DBS, whose viral load in whole blood is higher than 5000 copies/mL (Ct 33), is high. 2) CMV detection in DBS may be an alternative for CMV diagnosis in those situations in which blood extraction is difficult.

Keywords: citomegalovirus, dried blood spots, quantification.

P-135

Description of HPV genotypes in a population. Period 2009-2012

Marta Dominguez Gil*[1], Luz Ruiz[1], Carmen Ramos[1], Marta Arias[1], Ana García[2], José M. Eiros[1]

(1) Departamento de Microbiologia. Hospital Universitario "Rio Hortega". Valladolid (2) Departamento de Análisis Clínicos. HURH. Valladolid.

INTRODUCTION. In 2008, the Programme for the prevention and early detection of cervical cancer in Castilla-Leon, established a new strategy, which defined the screening tests, intervals and the target population group, and the structure guaranteeing the quality of the process. This program was aimed at healthy women sex 20-64 years. In the age range 20-34 years cytology performed every three years and in 35-64 years, cytology HPV detection and genotyping of five years.The aim of this study was to determine the prevalence of HPV infection in women included in the screening program, and describe the most common HPV genotypes in our environment.

METHODS. The laboratory of Microbiology, University Hospital "Rio Hortega", was assigned as one of

the centers of HPV determination within the program, referring samples from areas west of Valladolid and Segovia. During the 2009-2012, were processed for HPV detection, 35,355 samples. The HPV detection and genotyping technique was Clart ® HPV (Genomica)

RESULTS. Of the 35,355 samples analyzed by microarrays, a total of 3135 (8.9%) samples were positive for any HPV and 32220 (91.1%) were negative. In the 3135 samples positive for HPV genotype were analyzed in 2174 (69.3%) samples showed a single HPV genotype, whereas in 961 (30.7%) were documented coinfections. In analyzing the HPV detected by microarrays, we found that the most common genotypes were HPV 16 (15.2%), HPV 53 (11.7%), HPV 51 (10.3%), HPV 31 (10%), HPV 52 (9.1%), HPV 68 (8.9%), HPV 58 (8.5%) and HPV 66 (7.8%)

DISCUSSION. The highest percentage of HPV diagnostic concentrated in the 25-34 years, in which 19.4% of the samples tested positive for HPV screening. It was observed that with increasing age, diagnosis of HPV percentage linear decreasing trend with age, exactly as described in the general population screening. In this study the percentage of HPV 16 reached 15.2%, similar to that of other series in Spain, and others seem relatively common mucocutaneous infections that cause no transformants (HPV 11 and 6). As demonstrated in this study, no HPV 18 was the second most frequent genotype after HPV 16. HPV 16 was the most frequently detected genotype (15.2%). Genotype 53 is not included in any of the vaccines and in this study it was observed that the overall results of the genotyping was the second most common. Coinfection of multiple viral genotypes varied combinations forming together was a remarkable finding in this study

Keywords: HPV, epidemiology.

P-136

Clinical presentation of enteroviral meningitis: retrospective study 2009- 2012

Ana García*[1], Marta Domínguez Gil[1], Marta Arias[1], Carmen Ramos[1], Silvia Vega[1], José M Eiros[1]

[1] Departamento de Microbiología. Hospital Universitario "Río Hortega". Valladolid .

Introduction. The importance of viral infections of the central nervous system is not always well established. The availability of molecular detection techniques gives the clinical specificity that would otherwise not well cataloged. Our purpose is to describe a series of viral meningoencephalitis treated at a second level Spanish hospital over four years.

Methods. In the present study retrospectively reviewed charts of all documented Enterovirus meningitis in the microbiology laboratory of the University Hospital "Rio Hortega" of Valladolid, which serves a population of 250.000 people. We evaluated epidemiological, clinical and additional findings. Enterovirus detection was performed by molecular methods (SmartCycler, Izasa)

Results and Discussion. During the period 2009-2012 have been diagnosed 87 cases of enterovirus meningitis. Its prevalence has been steadily growing: 9, 10, 19 and 49 cases respectively over the four years. The proportion of male / female was 2/1, and 95% of patients were younger than 16 years. The seasonal distribution of cases showed a group of them (62%) in spring. The characterization allowed detection Echovirus 11 and 2 Coxsackie A Virus. All

patients had at least three major symptoms and signs of meningeal involvement.

As far as we know the present contribution describes a pioneering way in our Centre Enterovirus participation as agents of viral meningitis in children and adolescents. The possibility of detecting molecular diagnostic possibilities and expands its therapeutic management support, while allowing describe seasonal profile.

Keywords: meningitis, enterovirus, clinical.

P-137

Incidence of viruses in organic and non-organic crops of tomato and pepper in Valencia

Elena Lázaro[1], Carmen Armero[1], Josep Roselló[3], José J. Serra[3], María José Muñoz[4], Luis Rubio*[2]

[1] Department d'Estadística i Investigació Operativa. Universitat de València. Valencia [2] Dpto. de Protección Vegetal y Biotecnología. Instituto Valenciano de Investigaciones Agrarias. [3] Estación Experimental Agraria de Carcaixent. Instituto Valenciano de Investigaciones Agrarias. [4] Laboratorio de Virología. Generalitat Valenciana.C.A.P.A.A. Servicio de Análisis Agroalimentario. Valencia.

Organic agriculture is a production system that sustains the health of soils, ecosystems and people, relying on ecological processes, biodiversity and cycles adapted to local conditions. An important question is whether organic agriculture would be more or less vulnerable to diseases than non-organic intensive agriculture, particularly to those caused by viruses. We studied the incidence of viruses with regard to the organic and non-organic crops of tomato and pepper as well as other relevant factors such as plot management (green manure, adventitious plants, mulching, tillage practices, etc), diversity in time (crop rotation, organic fertilizers, traditional varieties, etc), in space (environment, adjacent plots, hedges, etc) and chemical fertilizers. A total of 30 plots in the Valencia province were classified according to the production system (organic or not) and the mentioned factors, and 240 individual plants (eight plants randomly selected in each plot) were observed for symptoms and analyzed for the presence of Cucumber mosaic virus (CMV), Tomato mosaic virus (ToMV) and Tomato spotted wilt virus (TSWV) by Enzyme-Linked ImmunoSorbent Assay (ELISA). The sample proportion of infected plants in the organic group was 0.21, nearly half of the observed proportion in the non-organic group, which was 0.41. Bayesian hierarchical logistic regression models were considered to perform the statistical analysis. Markov Chain Monte Carlo methods were used to approximate the posterior distribution of the model parameters and hyperparameters. These models suggest that organic farms are less susceptible to viruses, although more studies are necessary by analyzing more viruses and considering more geographical locations and different years to draw a conclusion since

virus epidemiology is a very dynamic and complex process.

Keywords: ecology, sustainability, agriculture, ToMV, CMV, TSWV.

P-138

Analysis of genetic and amino acid variation of the hemagglutinin (HA) of Yamagata and Victoria influenza B viruses circulating in Spain since the reemergence of Victoria lineage in late 90s

Unai Pérez Sautu*[1], Juan Ledesma[1]#, Francisco Pozo[1], Ana Calderón[1], Mónica Gonzalez Esguevillas[1], Mar Molinero[1], Inmaculada Casas[1]

[1] Influenza and Respiratory Virus Laboratory. National Center for Microbiology – ISCIII. Madrid.
#Equally contributed

Introduction: Two different evolutionary patterns of influenza B viruses (B/Yamagata/16/1988 and B/Victoria/2/87 phylogenetic lineages) can be found circulating among humans. They were identified during the 1987-88 season and underwent worldwide cocirculation until 1990, when Yamagata lineage became predominant. Victoria viruses did not re-emerged until late 90s. Despite the lack of subtypes, influenza B virus undergoes antigenic variation through genetic reassortment and antigenic drift from cumulative mutations. The HA is the major antigenic protein. The HA_1 domain within it contains all of the antigenic sites and is under continual immune-driven selection. Given that there is a lack of antigenic cross-reaction between the two lineages, vaccines including only one lineage confer limited or no protection against the opposite lineage strains and therefore, to correctly identify the predominant lineage likely to circulate in the upcoming season is of main concern to health protection authorities.

Objectives: This study describes the evolution of influenza B virus lineages in Spain during consecutive epidemic seasons between the years 1996 and 2012, based on the phylogenetic analysis of the nucleotide sequence of the HA and on the study of the amino acid change of the HA_1 domain.

Results and Conclusions: Circulation of Yamagata lineage viruses was dominant until 2002 when Victoria lineage emerged and started to cocirculate. Phylogenetic analysis indicates that Yamagata lineage has further diverged into two sub-lineages, while Victoria viruses have had only one evolutionary branch. Several amino acid substitutions have been identified in strains of both lineages at the epitope regions located in HA_1 domain. We have identified different sites within the antigenic loops and in the 190 helix which have undergone constant amino acid variation. We have also observed the same pattern in some residues which are not included in previously described epitopes. Altogether, this constant change pattern may reflect immunological selective pressure operating on those particular sites and thus, driving virus evolution.

Keywords: influenza B virus, Victoria, Yamagata, hemagglutinin.

P-139

Viropolis: The game for self-evaluation and learning Virology

Ana Doménech*[1], Laura Benítez[2], Mar Blanco[1], Mª Teresa Cutuli[1], Ricardo Flores[3], Juan García Costa[7], Josep Quer[4], Javier Romero[5], Antonio Talavera[6], Esperanza Gómez Lucía[1]

(1) Dpto. de Sanidad Animal. Facultad de Veterinaria Universidad Complutense de Madrid. (2) Dpto. de Microbiología III. Facultad de CC. Biológicas UCM. (3) Instituto Biología Molecular y Celular de Plantas. UPV-CSIC. Valencia (4) Laboratori de Malalties Hepàtiques. Hospital Universitari Vall D´Hebrón. Barcelona (5) Departamento de Protección Vegetal. INIA. Madrid. (6) Virología y Microbiología. Centro de Biología Molecular "Severo Ochoa" Madrid (7) Dpto. de Microbiología Hospital Cristal Piñor. Ourense.

Many students consider Virology as a subject to "memorize". A big challenge is to prepare specific materials that encourage students to broaden their knowledge of Virology, and, thus, become better professionals. Our team of university teachers/lecturers, researchers and doctors in hospitals has developed Viropolis, a computer game whose overall objective is for students to learn and test themselves about viruses and the diseases they produce, their treatment, diagnosis and prevention, and to enjoy learning about them. The possibilities of viral manipulation, and of viruses as agents in which to study different phenomena, or to be used as vaccine vehicles, are also included.

In the game, inspired in the well-known Monopoly, the player/s must protect a Community ("Viropolis") of viral infections. Players have to develop hospitals, laboratories, pharmaceutical companies and organizations to control and prevent viral diseases that affect humans, animals and plants. One or several players advance through a virtual heptagonal board distributed in cells, as indicated by the number shown in a virtual die. Every three consecutive cells belong to one of seven topics: "Human viral diseases", "Animal viral diseases", "Viral diseases of plants", "Research", "Diagnosis", "Antiviral drugs and vaccines," and "Prevention and control". Initially, players are given a number of points, which are increased or lost depending on their knowledge of viruses. The player who responds correctly to the question asked may buy the cell, if it is not owned by another player. If it has an owner, the newcomer must "pay" points to its owner for falling in it. Players know immediately if the answer is correct or not; an explanation that clarifies the solution is always provided.

The seven corner cells of the Board are occupied by "Start", "Epidemic area", "Course on quality of life", etc. where the player loses or gains playing turns. Interspersed in every three topic cells there is one "chance" cell, which either confers free points or penalizes the player, also through information on Virology. The player with the highest score wins the game.

Different types of questions are used: multiple choice, true-false, matching cards, relating the information in two columns, complete diagrams, or putting a puzzle together. This variety of activities, the high number of questions, and the competitive structure, make Viropolis very attractive for students.

Keywords: virology, education, computer game, learning, self-evaluation.

P-140

Spread of Enterovirus 68 among pediatric patients in Madrid, Spain

María Teresa Cuevas*[(1)], Francisco Pozo[(1)], Mar Molinero[(1)], Silvia Moreno[(1)], Cristina Calvo[(2)], María Luz García García[(2)], Begoña Santiago[(3)], Carmen Garrido[(3)], Jesús Saavedra[(3)], Unai Perez Sautu[(1)], Inmaculada Casas[(1)]

(1) Department of Influenza and Other Repiratory Viruses. CNM. Instituto de salud Carlos III. Majadahonda, Madrid (2) Pediatric Unit. Severo Ochoa Hospital. Leganés, Madrid (3) Pediatric Oncology Unit. Gregorio Marañón Hospital . Madrid.

Background: The genus Enterovirus (family Picornaviridae) contains 10 species, seven of them affect to humans: Enterovirus (EV) A-D, and Rhinovirus (RV) A-C. Enterovirus 68 (EV68) shares biological characteristics with rhinoviruses. EV68 was discovered in 1962 in respiratory samples. During the ensuing forty years, EV68 was rarely reported. In the last 10 years several EV68 outbreaks have been reported. Never before EV68 cases have been described in Spain. The phylogenetic analysis of EV sequences has allowed its classification into two lineages 1 and 2. Recently lineage 1 has been divided into two sub-lineages (1.1 and 1.2). Objectives: 1) To characterize EV positive respiratory samples in order to know if EV68 is circulating in Spain. 2) To develop a new real time PCR assay for enterovirus/rinovirus detection.Patients and Methods: During the influenza season 2012-13, an overall of 743 respiratory samples obtained from pediatric patients were analyzed, 720 from the Severo Ochoa Hospital (SOH) and 23 from the Gregorio Marañón Hospital (GMH). After nucleic acids extraction, two nested multiplex PCR assays were performed in order to detect 14 different respiratory viruses. Sequencing of EV, comprising VP4/VP2 partial coding regions, was done. Sequences were edited and analyzed using Chromas Lite 2.1 and Mega 5 softwares. Results: 1) An overall of 19 (2.6%) samples tested positive for EV, 10 of them (52.6%) were identified as EV68 (1 from GMH 9 from SOH). Seven sequences were included into sub-lineage 1.2 and three into lineage 2. Sub-linage 1.2: 6 sequences formed a monophyletic cluster, supported by 99% of bootstrap value. This cluster carried one signature amino-acid, A65V in VP4/VP2 sequence, which was no found in any of the EV68 sequences deposited in GenBank database. Another amino-acid signature was detected, K136R. The last sequence of this cluster grouped with the rest of the sequences with 80% of bootstrap value. Linage 2: Three sequences grouped within this linage, no specific amino-acid signatures were found. 2) A new real time PCR assay was successfully developed to detect Rhinoviruses and Enteroviruses, and it was implemented to the routine diagnosis in our laboratory. All the positive EV/RIN samples were confirmed by this real time PCR.Conclusion: This is the first report of the circulation of EV68 in Spain. The replacement of a conventional PCR for a real time PCR, allows a fast diagnosis of Enterovirus and Rhinovirus

Keywords: enterovirus 68, pediatric patients, real time PCR.

P-141

Problems of Norovirus detection in stool samples by antigen detection methods

Vanesa Mouro[1], Mirian Fernández Alonso[1], Marisol Escolano[1], Charo Remón[1], Patricia Sanz[1], Gabriel Reina[1]

(1) Department of Clinical Microbiology. Clínica Universidad de Navarra. Pamplona.

Introduction. Norovirus (NoV) is a leading causative agent of gastroenteritis worldwide and is often involved in outbreaks in communal facilities. There are six recognized NoV genogroups, and three of them (GI, GII, and GIV) affect humans. The aim of this study was to compare the detection of NoV antigen (Ag) with the detection by molecular techniques.

Methods. A total of 79 stool samples were collected prospectively from October to December 2012, among patients with gastroenteritis attended in our hospital. Patients age median was 43 (IQR:11-63). RT-PCR for NoV detection was performed over every sample, while Ag detection was carried out with 63 samples, as the remaining where originally studied for *Clostridium difficile* toxigenicity, but not for virological diagnosis. NoV Ag was detected using the RIDA®QUICK Norovirus test (R-Biopharm).RT-PCR for NoV detection was carried out with an in-house conventional multiplex method. RNA was obtained from stools by manual extraction of diluted and 0,2 µm filtered samples with the High Pure Viral Nucleic Acid Kit (Roche). A modified version of the RT-PCR protocol described by Kageyama et al (2003) was used for detection of GI and GII Noroviruses.

Results. Twelve out of 79 samples studied by RT-PCR had a positive result (15,2%), while 10 out of 63 (15,9%) stools studied by Ag detection were considered positive.A total of 18 discrepancies were found among the 63 samples in which both techniques were carried out. These discrepancies were probably due to an incorrect performance of the Ag detection test as different results were found depending on the way the samples were placed into the membrane cassette. Therefore,a total of 9 false positive results could have been obtained by using this methodology. The remaining 9 discrepancies (positive result by RT-PCR and negative by Ag detection) could be due to a greater sensitivity of the molecular technique, as no other pathogens were found.

Among the 16 stool samples in which viruses were not investigated, two cases showed a positive RT-PCR result.Patient ages where essentially under 2 or over 60 years. All the positive tests corresponded to GII NoV strains (the most prevalent genogroup in Spain).

Conclusions. Norovirus detection should be carried out by molecular techniques as antigen detection shows low sensitivity and is susceptible to variation regarding its performance. This pathogen should be always ruled out as a gastroenteritis possible etiology in extreme ages.

Keywords: norovirus, RT-PCR, gastroenteritis.

P-142

Diagnosis of congenital Cytomegalovirus infection by DNA detection in dried blood spots

Mirian Fernández Alonso[1], Gabriel Reina[1], Juan Narbona[2], Valentín Alzina[2], Laura Moreno[2], Sada Zarikian[2]

(1) Department of Clinical Microbiology. Clínica Universidad de Navarra. Pamplona (2) Departments of Pediatrics. Clínica Universidad de Navarra. Pamplona.

Introduction. Cytomegalovirus (CMV) detection in clinical samples taken within the first 2-3 weeks after birth differentiates congenital CMV infection from the postnatal acquisition of this virus. Recently, it has been suggested the possibility to use dried blood spots (DBS), collected at that age, to diagnose congenital CMV infection retrospectively. In this study we discuss the performance of CMV DNA detection from DBS.

Methods. We studied 17 patients with suspicion of congenital CMV infection due to hearing loss or CMV detection in clinical samples within the first months of life. Average patients age was 12 months (SD: 13).

The procedure of CMV DNA detection in DBS includes two separate steps. First, DNA extraction from DBS was performed by cutting approximately 1cm^2 of DBS, in which an estimated blood volume of 30µL was present. This was followed by digestion with ATL buffer (QIAGEN) and Proteinase K during one hour, and manual extraction with the High Pure Viral Nucleic Acid Kit (Roche). Secondly, real time PCR (rt-PCR) was carried out in LightCycler 2.0 (Roche) with the LightCycler® CMV Quantitative Kit (Roche).

Results. CMV DNA was detected in 4 out of 17 patients investigated (23,5%), containing the positive samples an average CMV viral load of 10200 copies/mL.

This technique confirmed in four patients the presence of CMV in blood samples collected within the first days of life, so a congenital CMV infection could be diagnosed in them. The limit of detection, using this rt-PCR method from DBS, resulted in approximately 1250 copies/mL.

Conclusions. Cytomegalovirus DNA detection is an easy method which can be performed by any conventional clinical laboratory where molecular techniques are carried out. This method is useful for confirmation of congenital CMV disease.

Keywords: cytomegalovirus, cried blood spots, congenital Infection.

P-143

Serological study of a mumps outbreak. Detection of neutralizing antibodies to circulating strain

Mercedes Rodríguez[1], Óscar Martínez[1], Susana Rojo[1], Pilar Leiva[1], Carmen Díaz Carrio[1], Gerardo Cuesta[1], José A Boga*[1]

(1) Servicio de Microbiología. Hospital Universitario Central de Asturias. Oviedo.

Objectives: 1) A serological study of a mumps outbreak was performed. 2) To test if mumps-specific antibodies detected in sera of people born after 1981 and vaccinated with a type A-strain, neutralize the strain circulating in this outbreak.

Patients and methods. 836 serum samples belonging to 812 patients (458 women, mean age 24.36 ± 13.6, median 21, range 1-96) with suspected mumps (8 patients had orchitis and 1 pancreatitis) were collected during a mumps outbreak in Asturias in 2012. Two serum samples were available in 24 patients and a single sample in 788 cases. Anti-mumps virus IgGs and IgMs were detected by immunosorbent assay. In Asturias, routine vaccination against mumps began in 1981 maintaining vaccine coverages of more than 90% since 1993.

Neutralization tests were carried outto examine the presence of virus neutralizing antibodies and to measure antibody titers.

Results. Mumps was diagnosed in 197 (24.2%) patients [185 with positive IgM and 12 with a significant rise of IgG]. The mean age of positive patients was 27.3 ± 17.8, median 30, range [8-57]. Primo-infection was present in 9 patients. The 76.3% of those born after 1981 had no detectable IgM titer despite having symptoms of mumps. The median serum antibody titer in each group is:

	Group A	Group B	Group C	Group D
	(n=11)	(n=5)	(n=5)	(n=8)
Median	64	512	32	32
Mínimum	8	256	8	16
Máximum	2048	2048	256	128

Group A: Positive IgM and negative IgG serum
Group B: Positive IgG and negative IgM serum - Positive PCR

Group C: Positive IgG serum of uninfected born after 1981

Group D: Positive IgG serum of uninfected born before 1981

Conclusions**.** 1) Serological methods are useful to diagnose a large number of cases of mumps. 2) A high percentage of mumps infected patients, who are old enough to be vaccinated, had no detectable IgM titer. This fact could be explained because of the development of a secondary immune response. A second serum sample is necessary to confirm these cases. 3) Sera from vaccinated individuals (Group C) as well as from those with mumps immunity due to natural disease (Group D) have antibodies that neutralize the strain circulating in this outbreak. 4) In acute infection, serum antibody titer of patients with a secondary immune response due to IgG antibodies (Group B) is higher than that of patients with primo-infection (Group A).

Keywords: mumps virus, vaccination, neutralization.

P-144

Genomic cuantification of RSV. Relationship of viral load and clinical manifestations

Ana Morilla*[1], José A Boga[1], Óscar Martínez[1], Marta E. Álvarez Argüelles[1], Julián Rodríguez[2], María de Oña[1], Santiago Melón[1]

(1) Servicio de Microbiologia. Hospital Universitario Central de Asturias.Oviedo (2) Servicio de Pediatría. Hospital Universitario Central de Asturias. Oviedo.

Objective: Design and standardization of a method for diagnosis of RSV based on genome quantification and study of the meaning of the viral load.

Patients and methods: 114 RSV positive samples (60 nasopharyngeal swabs, 30 pharyngeal swabs, 23 nasal swabs and 1 tracheal aspirate) from 107 patients were quantified by real time-PCR. In 73 samples, serial 10-fold dilutions of two plasmids containing RSVA or RSVB amplicons were used as standard curve. In the remaining samples, quantification was accomplished via extrapolation from an external standard curve.

Results: Table 1. Cycle threshold (Ct) of serial 10-fold dilutions of RSVA and RSVB plasmids

	copies	5x106	5x105	5x104	5x103	5x102	50
pRSVA	Median Ct	20	22.1	25.9	29.3	32.1	35.7
	SD	1.89	2.2	2.53	1.55	1.72	1.15
	N	20	19	17	14	10	3
	IC 95%	19.1-20.9	21.0-23.2	24.6-.27.2	28.4-30.2	30.8-33.3	32.8-38.5
pRSVB	Median Ct	22.2	25.7	27.8	32.5	35.2	37
	SD	1.8	1.8	3.7	1.2	1.1	0
	N	17	20	16	13	6	1
	IC 95%	21.2-23.1	24.8-26.6	25.7-29.7	31.7-33.2	33.9-36.4	37

Table 2. Relationship of RSV viral load in different samples and age, clinical manifestation and hospitalization

	Pharyngeal (n)	Nasal-nasopharyngeal (n)
<1 year	6.17±1,32 (10)	6.12±1,56 (68)
1-4 years	5.58±1,64 (12)	5.63±1,82 (12)
>4 years	3.84±2,26 (8)	5.58±2.38 (3)
p	0.0247	ns
Bronchiolitis	5.84±1,19 (7)	6.34±1.48 (55)
Respiratory dis.	6.01±1,84 (8)	5.41±2.24 (13)
Pneumonia	3.29±2,58 (5)	5.42±1.14 (7)
Cold-like symp.	5.39±1,55 (10)	5.39±1.32 (8)
p	<0.05	ns
Hopitalized	5.41±1,82 (11)	6.14±1.58 (41)
No Hopitalized	4.85±2,21 (13)	5.88±1.54 (22)
p	ns	ns

Conclusions: 1) The reproducibility of our assay using RSV A and RSV B plasmids appears to be high. 2) Viral load was lower in pharyngeal swabs of the patients older than 4 years old, suggesting a less active replication in that localization 3) Viral load was lower in pharyngeal swabs in patients with pneumonia 4) There are no differences in the viral load between hospitalized and not hospitalized patients.

Keywords: respiratory syncityal virus, viral load, clinical manifestations.

P-145

Detection of gastrointestinal viruses. Analysis of external quality control SEIMC

Nieves N Orta[1,4], Mª del Remedio R. Guna[2,4], Enrique E Ruiz de Gopegui[3,4], Marta M Poveda[4], María M Ovies[4], José Luis Pérez[3,4], Concepcion C. Gimeno*[2,4]

(1) Servicio Microbiología. Hospital Francesc de Borja. Gandia, Valencia. (2) Consorcio Hospital General Universitario.Valencia (3) Servicio de Microbiología. Hospital Son Espases. Palma de Mallorca. (4) Control Externo de Calidad. SEIMC. Madrid .

Objective:To evaluate the results obtained in the External Quality Control of the Spanish Society of Clinical Microbiology and Infectious Diseases in the detection of gastrointestinal viruses.

Material:From 2001-2012 six shipments were sent to average of 77 participating centers and comparing their results with those of a reference laboratory. Samples were faeces and were accompanied by a clinical history, asking for the detection of viruses that cause diarrhoea. Each sample was positive for the following viruses: Rotavirus Astrovirus, Adenovirus and Norovirus.

Results:The centers participating varied with the years, 61.7% in 2001 to 87.5% in 2012. In regard to the viruses causing diarrhoea is observed that when dealing with rotavirus or adenovirus more than 97% of the centers performe relevant tests to detect them, but when astrovirus or norovirus are the viruses causing diarrhoea only 23.7% and 59.3% of the centers performe the tests, respectively. As success rate shows that in rotaviruses it passes from 73% in 2001 to 100% in 2007 and 2012, in the astrovirus was 83.3%, 88.5% in adenovirus and in norovirus of 45.9%. Diagnostic methods used in a majority were immunochromatography techniques (IC), latex agglutination (LA) and enzyme immunoassay (EIA). EIA techniques obtained a 100.0% of correct results in the control of rotavirus, 81.8% for astrovirus, 92.2% for adenovirus and 20.0% for norovirus. Finally, LA obtained the results: 73.3%, 100%, were not employed in astrovirus and norovirus, and 33.3% in adenovirus. Significantly astrovirus was detectable only by PCR and EIA and, in the case of norovirus, the most common techniques and the best results were with molecular microbiology (with IC obtained false negative results in all cases).

Conclusions:There's an increase of participation in this Program. There's an increase of centers that detect viruses in faeces, with greater participation in Rotavirus and Adenovirus. Viruses with poorer detection rates are norovirus and astrovirus. Overall, EIA techniques obtained good results in detecting all viruses except norovirus, the IC obtained good results in rotavirus and adenovirus but not used for astrovirus and in norovirus don´t get any positive results. When using molecular diagnostic techniques are usually obtained good results in all cases. These results show that few laboratories registered are well trained to detect astrovirus and norovirus.

Keywords: external quality control, gastrointestinal viruses

P-146

A novel methodological approach for enteric virus detection in food supply chains

David Rodríguez Lázaro*[1], Nigel Cook[2], Marta Diez Valcárce[3], Marta Hernández[1]

(1) Department of Food Safety. Instituto tecnológico Agrario de Castilla y León.Valladolid (2) Department of Food and Envirenonmental Microbiology. Food and Envirenonmental Research Agency (FERA).York UK (3) Departamento de Biotecnología y Tecnología de los Alimentos. Universidad de Burgos.

The number of viral foodborne outbreaks has increased in the last years. Molecular methods, particularly real-time PCR, have become the standard for detection of viruses in food supply chains, as they are the optimal techniques for this purpose. Within the frame of the EU project VITAL (www.eurovital.org) the detection of relevant foodborne pathogenic viruses and agents demonstrating an existent route of contamination (hepatitis E virus, hepatitis A virus, human norovirus genogroup I and II and human and porcine adenovirus) was performed using real-time RT-PCR. This project has devised a methodological strategy divided in 3 different phases: extraction and concentration of virus from the matrix, the isolation of the viral nucleic acid, and its amplification using (RT) real time-PCR. The novelty of the approach also included that a set of controls were added in each phase to guarantee the reliability of the results: (i) sample process control virus (SPCV) was added during the viral elution and concentration from the food surface, (ii) the negative extraction control (NEC) was included during the viral nucleic acid extraction and, finally, (iii) the Internal amplification control (IAC) and positive and negative amplification controls (PAC and NAC, respectively) were used to assess the performance of the (RT)-real-time PCR. For those virus types that cannot be or are very difficult to propagate in cell culture, chimeric nucleic acids were synthesised and their applicability as PACs demonstrated.

To harmonise the use of VITAL strategy within the 11 labs involved in the project and as an example for further international implementation, a total of 23 standard operating procedures (SOPs) were written, and one of the methods, that for detection of adenovirus in soft fruits, was internationally evaluated by means of a ring trial exercise. This is the first example of a harmonised international effort for validation of molecular methods for detection of enteric viruses in foods.

Finally, sampling studies were performed using this methodological strategy in three European food supply chains: pork production, shellfish and vegetables. A total of 1543 food and food-related samples were analysed in nine European countries by nine different laboratories using an iden-

tical harmonised approach, which guaranteed a rapid and comparable interpretation of the results.

Keywords: virus, detection, real-time PCR, food.

P-147

Drift to jump: transition towards viral genome segmentation prompted by point mutations

Elena Moreno*[1], Samuel Ojosnegros[2], Juan García-Arriaza[3], Esteban Domingo[1,4], Celia Perales[1,4]

(1) Department of Virology. Centro de Biología Molecular Severo Ochoa. Madrid (2) Departmento of Biology. California Institute of Technology. Passadena, USA (3) Department of Virology. Centro Nacional de Biotecnología. Cantoblanco, Madrid.
(4) Departamento de Hepatología. Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd) Spain

The possible requirement of point mutations for drastic evolutionary transitions such as those mediated by recombination is an unsolved issue. The important animal picornavirus foot-and-mouth disease virus (FMDV) offered a unique opportunity to address this question. Upon more than 200 serial passages of the biological FMDV clone C-S8c1 in BHK-21 cells at high multiplicity of infection, the virus accumulated multiple point mutations scattered throughout the genomes, and underwent a transition towards genome segmentation. This virus, termed C-S8p260, lacked detectable standard (full-length) genomes, and infected and killed cells by complementation of two genomic forms, each including an in-frame deletion. One deletion spanned 417 nucleotides within the L (leader protease-coding region) and the corresponding RNA is termed 417 RNA. The other deletion spanned 999 nucleotides within the capsid-coding region, and the RNA is termed 999 RNA. We addressed the question of whether the point mutations accumulated in 417 and 999 RNAs were a requirement for the evolutionary transition towards segmentation. To this aim we constructed plasmids expressing FMDV RNAs with the deletions in the context of either the parental, C-S8c1 or the evolved C-S8p260. Co-electroporation of BHK-21 cells with mixtures of segmented FMDV RNAs showed a dramatically higher polyprotein expression and infectious progeny production when the internal deletions were placed under the sequence context of the FMDV C-S8p260, as compared with the sequence context of C-S8c1. Moreover, efficient complementation between segmented RNAs was dependent not only on point mutations but also on the precise location of 417. A minimal displacement of the 417 deletion three nucleotides either downstream or upstream in the viral RNA decreased drastically the complementation activity of the RNA. This observation suggests that only a subset of many possible deletions is compatible with high fitness of the segmented genome version. The results with FMDV suggest that remarkable recombination-mediated transitions in RNA viruses may necessitate underlying, fitness-enhancing point mutations. For general evolution, the requirement

of point mutations for a major evolutionary transition opens the interesting possibility that gradual evolution can be a prerequisite for drastic genetic alterations, and that gradualism and punctuated equilibrium may be linked evolutionary processes rather than opposed evolutionary models.

Keywords: defective genomes, evolution, sequence context.

P-148

Viruses and the RNA world

Fabián Reyes Prieto[1], Ricardo Hernández Morales[1], Rodrigo Jácome[1], Arturo Becerra*[1], Antonio Lazcano[1]

(1) Departamento de Biología Evolutiva. Facultad de Ciencias, UNAM. México DF.

A detailed bioinformatic search for ribonucleotidyl coenzyme biosynthetic sequences in DNA- and RNA viral genomes are presented. No RNA viral genome sequence appears to encode for sequences involved in the coenzyme biosynthesis. In both single- and double-stranded DNA viruses a diverse array of coenzyme biosynthetic genes has been identified, but none of the viral genomes examined here encodes for a complete pathway. Our results do not support the possibility that RNA viruses are direct holdovers from an ancient RNA/protein world. Extrapolation of our results to evolutionary ages prior to the emergence of DNA genomes suggests that during those early stages living entities may have depended on discontinuous genetic systems consisting of multiple small-size RNA sequences.

The discussion and results presented here, were published in *Biochimie.* 2012 Jul;94(7):1467-73.

Keywords: RNA world, virus evolution.

P-149

Modelling viral evolution and adaptation: challenges and rewards

Susanna Manrubia[1]

(1) Department of Molecular Evolution Centro de Astrobiología (INTA-CSIC) Torrejón de Ardoz, Madrid.

Viral populations are extremely plastic. They maintain and steadily generate high levels of genotypic and phenotypic diversity that may result in different adaptive strategies. A major unknown factor in constructing realistic models of viral evolution is how mutations affect fitness, which amounts to unveiling the nature of viral fitness landscapes. Our understanding of viral complexity is improving thanks to new techniques as deep sequenc-

ing or massive computation, and to systematic laboratory assays. In this way, we are clearing up the role played by neutral networks of genotypes, by defective and cooperative interactions among viral mutants, or by coevolution with immune systems. Models of viral evolution are thus improving their accuracy and becoming more competent from a conceptual and a predictive viewpoint.

The adaptive strategies of viruses make up a large, innovative, and ingenious ensemble of mechanisms that defeat our most creative expectations. A shallow overview of some of their remarkable features confronts us with many open questions in the way their populations change and adapt, as well as with the processes and mechanisms one should in principle consider in realistic models of viral evolution. Viruses, especially those with an RNA genome, perform broad explorations of genome space due to their relatively short genomes, large population sizes, and elevated mutation rates. Their dynamics are conditioned by the existence of neutral networks of genotypes (producing the same phenotype) that span genome space in a likely variable, and as yet mostly unknown, extent. In establishing the relation between genotype and phenotype, it is critical to conceive fitness landscapes that capture the essentials of how genomic changes affect function. Viruses generate diversity through point mutations, but also through major modifications as segment deletion, non-homologous recombination or segment shuffling. In those conditions, many viruses produce a large amount of defective genomes that may thrive in appropriate environments thanks to the complementation offered by complete genomes. These characteristics and several others are behind the response of pathogenic viruses to therapies, and should be taken into account when designing protocols to control the spread of viral infections.

S. C. Manrubia, *Current Opinion in Virology*- 2012, 2:531:537

Keywords: viral evolution, modelization, fitness landscape, adaptive strategy.

P-150

Experimental evolution of genome architecture and complexity in RNA virus

Anouk Willemsen*[(1)], Eszter Majer[(2)], Zaira Salvador[(2)], Mark P Zwart[(1)], José Antonio Daròs[(2)], Santiago F. Elena[(1,3)]

(1) Evolutionary Systems Virology Group. Instituto de Biología Molecular y Celular de Plantas (CSIC-UPV). Valencia (2) Department of Plant Virus Biotechnology. Instituto de Biología Molecular y Celular de Plantas (CSIC-UPV) Valencia.

Experimental evolution has led to several breakthroughs in evolutionary biology: confirmation of hypotheses on adaptive trajectories, the role of population size and chance events, the benefits of sex, evolvability, and evidences for coevolution. One aspect of evolution that has received scant attention, however, is the evolution of genome complexity: the dimensions and organization of the genome. Although some theoretical and *in silico*studies have addressed this topic, very little ex-

perimental work has been reported. We are studying the evolution of genome architecture with *Tobacco etch potyvirus*, a plant RNA virus. We are exploring the evolutionary dynamics of both decreasing an increasing genome size and complexity by duplications of existing genes, incorporation of new genes, changes in gene order, and segmentation of the viral genome.

First, we chose to alternate the position of the NIb cistron to all possible alternative positions in the genome. All known *Potyviruses* conserve gene order, and we therefore wanted to know whether it is possible to reorder the genome and what the ramifications for the evolutionary potential of the virus will be. Constructs were tested for viability in wildtype and transgenic plants expressing NIb. Only two out of nine constructs were viable; they incorporated NIb 5' of P1 or between P1 and HC-Pro cistrons. These two viruses have markedly reduced infectivity and fitness, even after they have been evolved for a number of serial passages. Second, we give two other examples of our ongoing work: (*i*) addition of heterologous silencing suppressor (*Cucumber mosaic cucumovirus* 2b) to allow the multifunctional protein HC-Pro to specialize in other functions, and (*ii*) duplication of homologous cistrons (NIb, NIa-Pro and CP) to test whether they can enhance viral fitness. Illumina NGS will be used to map changes in the viral genome, and we will quantify fitness for evolved lineages of all these viruses.

Keywords: experimental evolution, virus, genome architecture, complexity.

P-151

Competition among beneficial mutations leads to the coexistence of multiple polymorphisms in bacteriophage Qß evolved at increased error rate

Laura Cabanillas*[(1)], María Arribas[(1)], Ester Lázaro[(1)]

(1) Departamento de Evolución Molecular. Centro de Astrobiología. (CSIC-INTA) Torrejón de Ardoz, Madrid .

When beneficial mutations present in different genomes spread simultaneously in an asexual population, their fixation can be delayed due to competition among them, which is known as dynamics of interference. The intensity of interference is determined by the rate of beneficial mutations, which in turn depends on the population size, the total error rate, and the degree of adaptation of the population. RNA viruses, with their large population sizes and high error rates, are good candidates to present a great extent of interference. To test this hypothesis, we have investigated whether competition among beneficial mutations was responsible for the prolonged presence of polymorphisms in the mutant spectrum of an RNA virus, the bacteriophage Q , evolved in the presence of the mutagenic nucleoside analogue 5-azacytidine (AZC) during a large number of generations. The analysis of the mutant spectra of virus populations isolated at different points of the evolutionary series showed that polymor-

phisms distributed into several evolutionary lines that can compete among them. Each evolutionary line can provide a selective advantage by a different molecular mechanism leading to the coexistence of multiple adaptive pathways in the population. The presence of accompanying deleterious mutations, the high degree of recurrence of the polymorphic mutations, and the occurrence of epistatic interactions generate a highly complex interference dynamics, making it difficult the emergence of a defined consensus sequence (Cabanillas et al., 2013). Our results show that interference can also be seen as a positive factor that allows the exploration of the different local maxima that exist in rugged fitness landscapes.

Keywords: RNA viruses, Interference, beneficial mutations, mutagenesis, 5-azacytidine, polymorphisms.

P-152

Holding on to the future: experimental evolution of pseudogenization in viral genomes

Mark P. Zwart*[1], Anouk Willemsen[1], José Antonio Daròs[1], Santiago F. Elena[1]

(1) Instituto de Biología Molecular y Celular de Plantas. CSIC-UPV . Valencia.

Viruses have evolved highly streamlined genomes and a variety of mechanisms to compress them, suggesting that genome size is under strong selection. Horizontal gene transfer has, on the other hand, played an important role in virus evolution. There is therefore a paradox: heterologous elements that are initially nonfunctional cannot be acted upon by evolution and functionally integrated into the host genome if they are rapidly purged by selection against increased genome size.

Here we report on the experimental evolution of pseudogenization in virus genomes using a plant RNA virus (*Tobacco etch virus*; family *Potyviridae*) expressing GFP in between viral P1 and HC-Pro cistrons. The virus was evolved in tobacco plants for 27 weeks in total, with a passage length of one, three or nine weeks. End-point populations were subjected to NGS with Illumina and we determined their within-host fitness, accumulation and virulence. At the end of the experiment, a complete loss of GFP fluorescence was observed in all ten 9-week lineages and in two out of ten 3-week lineages. Only a partial loss of fluorescence was observed in a single 1-week lineage. Large genomic deletions, encompassing GFP and sometimes the 5´ end of HC-Pro cistron, were found in all lineages that lost fluorescence. The within-host fitness of lineages with genomic deletions was markedly improved, although virulence and accumulation of all lineages had not significantly changed. The 3-week lineages without genomic deletions had a higher fitness than 1-week lineages and the ancestral virus, and analysis of SNPs revealed considerable convergent evolution in the 3- and 9-week lineages with genomic deletions.

Our results show that the strength of selection for a reduced genome size and the rate of pseudoge-

nization depend on environmental conditions (i.e., length of passage time). Moreover, in our model system there is clearly an inverse correlation between genome size and within-host fitness. Finally, our results shed light on how evolution can act to integrate heterologous elements into virus genomes. For the three-week passage condition, selection is clearly acting on the virus populations and increasing their fitness, whilst it is not strong enough to immediately remove the heterologous element. A "sweet spot" therefore exists were the strength of selection is just right to allow for the opportunity that evolution might integrate a foreign gene into the genome rather than purge it.

Keywords: genome evolution, pseudogenization, fitness, plant virus.

P-153

Some ecotypes of *Arabidopsis thaliana* favor local adaptation of Tobacco etch potyvirus while others select for generalist viruses

Julia Hillung*[(1)], José Manuel Cuevas Torrijos[(1)], Santiago F. Elena[(1,2)]

(1) Instituto de Biología Molecular y Celular de Plantas. Consejo Superior de Investigaciones Científicas. Universidad Politécnica de Valencia. (2) The Santa Fe Institute. Santa Fe, USA.

In this study we sought to evaluate to what extent the genetic diversity of the host conditions the evolutionary fate of a plant RNA virus. To this end we performed a 3-fold replicated evolution experiment in which *Tobacco etch potyvirus* isolate *At*17b (TEV-*At*17b), previously experimentally adapted to *A. thaliana* ecotype L*er*-0, was serially passaged in a set of five genetically heterogeneous ecotypes of *A. thaliana* partially susceptible to infection. Passages were carried out every three weeks. After 15 passages we found that adapted viruses showed higher infectivity and stronger virulence on their local ecotypes. Full-genome consensus sequences were obtained and host-specific and convergent mutations among lineages were observed.

Next, we sought for the specificity of virus adaptation. To this end, we infected all five ecotypes with all 15 evolved virus populations. The result of this factorial infection was characterized phenotypically (infectivity, virulence, accumulation, and symptoms development). We constructed an ecotype-by-virus population fitness heat map to better visualize the results. We found that some ecotypes were more permissive to infection than others, and that some virus populations were more specialist/generalist than others.

We conclude that (i) the small genetic differences existing among host ecotypes were enough to select for differences in viral fitness traits, (ii) genetic variation exist in TEV-*At*17b to allow for divergence in response to differences in host genetic background and that the response to selection may be either local adaptation (specialization) or unspecific (generalism), and that (iii) experimental evolution is a powerful tool to modify the way a plant RNA virus and its host interact.

In ongoing transcriptomic profiling experiments we sought to discover whether observed differences in viral fitness traits correlate to differences in the way the different plant ecotypes respond to infection.

Keywords: experimental evolution, tobacco etch potyvirus, plant viruses.

P-154

miRNAs involved in insulin resistance are regulated *in vitro* by HCV infection

Jose A. del Campo*(1), Marta García-Valdecasas(1), Ángela Rojas(1), Manuel Romero-Gómez(1)

(1) UCM Digestive Diseases. Valme University Hospital & CIBERehd. Sevilla.

Background and aims: The hepatitis C virus induces insulin resistance and steatosis, but the molecular mechanisms involved in these processes are not yet completely understood. PTEN is an inhibitor of PI3K protein which plays an essential role in the insulin signaling pathway. PTEN is down-regulated in infected cells. The aim of this work is to analyze the mechanism in which JFH1 interacts with PTEN and PI3K, and with others downstream proteins of the insulin signaling pathway, studying the expression of miRNAs targeting PTEN (miRNA29a and b), and the role of a PI3K inhibitor (LY294002) in viral replication.

METHODS: Huh7.5 cells were grown and infected with the JFH1 replicon (1 particle/cell). LY294002 (10μM) or insulin (10nM) were added to the cells. Total RNA extraction was performed 48 hours after the media was changed. The expression of the different genes was quantified using the qRT-PCR Quantace (Bioline) kit and analysis of specific proteins analyzed by Western-Blot. miRNA expression was quantified using the miScripReverseTranscription and miScript SYBR®Green commercial Kits (Quiagen).

RESULTS: Huh7.5 cells infected with JFH1 showed decreased PTEN and PI3K protein levels. In cells infected and with hyperinsulinemia, PTEN was further decreased whereas PI3K showed no difference compared to control experiments. When Huh7.5 cells were treated with the PI3K inhibitor LY294002, gene expression of PI3K was increased in control 1.6 (±0.3) and in virus 2.6 (±0.9) fold induction respectively, likely due to a feed-back cellular response. AKT and mTOR, downstream proteins of the PI3K pathway, are increased in control and infected cells treated with LY294002 (AKT: 1.7±0.7 vs. 1.6±0.01 fold induction; mTOR: 1.5±0.1 vs. 2.6±0.8 fold induction). Other proteins of the insulin signaling pathway PTEN4, PTP1B and PTP2 showed increased gene expression. LY294002 inhibitor did not affect significantly viral replication rates (decreasing by 20% compared to -interferon). miRNA29a and 29b (targeting PTEN), were increased 1.5 (±0.01) and 2.1 (±0.1) fold induction in cells infected with JFH1.

CONCLUSION: PI3K inhibitor LY294002 modulates gene expression and slightly decreases viral replication, probably due to inhibition of IRS1 phos-

phorylation. miRNA29a and 29b induction could explain the reduction of PTEN levels and seems to be a link between HCV infection and metabolic disorders.

Keywords: miRNAs, HCV, insulin resistance, viral replication.

P-155

Molecular epidemiology of hepatitis Delta virus. First data of Spain

Óscar Crespo*[1], José Manuel Echavarría[2], Lucía Morago[1], María del Carmen García-Galera[1], Silvia Calleja[1], Ana Avellón[1]

(1) Hepatitis Unit. National Centre of Microbiology. Majadahonda, Madrid (2) Virology Area. National Centre of Microbiology. Majadahonda, Madrid.

Introduction and Objective: Hepatitis Delta virus (HDV) causes liver disease in previously hepatitis B virus (HBV) infected patients. The virus is defective and needs a helper function provided by the HBV surface antigen. The prevalence of HDV infection varies according to the geographical area being estimated around 15 million HDV carriers worldwide. The 1679 nt genome of the HDV is highly diverse, aspect that make technically difficult its molecular analysis. HDV is currently classified into eight genotypes (G) named I to VIII. At present there are few phylogenetic studies, being few in Europe and none in Spain (SP). HDV G-I is considered worldwide distributed. The objective of this work is thestudy of HDV phylogeny and molecular epidemiology in Spain for the first time.

Materials and methods: a new HDV all-genotype amplification system was designed. After amplification (nested PCR) and sequencing (Sanger method) of a 794 nt fragment, about 750 nt including the hepatitis delta antigen carboxyl terminal end (100 amino acids) were analyzed. A total of 64 RNA extracts from HDV PCR positive samples received in the National Center of Microbiology between 2007 and 2012 were included. The nucleotide sequences of HDV isolates obtained were compared to 76 previously reported HDV sequences retrieved from GenBank (31 G-I, 11 G-II, 4 G-III, 16 G-IV, 6 G-V, 3 G-VI, 2 G-VII and 3 G-VIII). MEGA 5.1 software was used for alignment and phylogenetic tree construction (neighbor joining method, 1000 bootstrap re-sampling test).

Results: phylogenetic analysis was able to differentiate all 8 genotypes in separated clusters with the following bootstrap values (BV) of the branches: G-I (BV=76%), G-II (BV=90%), G-III (BV=100%), G-IV (BV=99%), G-V (BV=99%), G-VI (BV=99%), G-VII (BV=88%) and G-VIII (BV=99%). A total of 63 isolates tested corresponded to G-I, while the remaining was G-V. The analysis of G-I sequences revealed 4 clusters with 99% BV that include 3 [SP 2008-2011 (n=2) and USA 1996], 12 [SP 2010-2012 (n=11) and Romania 2007], 11 [SP 2010 and China-Taiwan-Japan 1994-2010 (n=10)] and 19 [SP 2007-2010 (n=13), France 2007 (n=3), Italy-Romania- USA 2006-2007] sequences respectively.

Conclusions: the new method was able to amplify both GI and GV, which can be considered divergent each other. G-I revealed to be the most prevalent in Spain. G-I presents high diversity with clustering that may reflect the complex epidemiology distribution of HDV worldwide.

Keywords: delta, hepatitis virus, molecular epidemiology.

P-156

Generation of permissive BCLC5 cell lines for the study of HCV replication: role of miR122 in replication enhancement

Mairene Coto*[1], George Koutsoudakis[1], Loreto Boix[2], Juan Manuel López Oliva[2], Carlos Fenández Carrillo[1], Patricia Gonzalez[1], Jordi Bruix[2], Xavier Forns[1], Sofía Pérez del Pulgar[1]

(1) Liver Unit. IDIBAPS, Hospital Clinic, CIBERehd. Barcelona (2) BCLC group, Liver Unit. IDIBAPS, Hospital Clinic, CIBERehd . Barcelona.

Robust hepatitis C virus (HCV) cell-culture propagation is restricted almost exclusively to the human hepatoma cell line Huh7 and its selected subclones, which are highly permissive for HCV replication; e.g., Huh-7.5 cells. Recent studies suggest that host factors such as innate immunity, gene polymorphisms, and/or miR122 are associated with the permissiveness of cells for HCV infection. We have recently characterized the BCLC5 cell line, which is derived from human hepatocellular carcinoma, and which can support subgenomic (SGR) HCV RNA replication for prolonged periods of time. In order to obtain highly permissive cell lines for HCV replication, clonal BCLC5 cells-(designated BCLC5-C8 cells) harboring a selectable neomycin (neo)-SGR were cured of HCV RNA with IFN-alpha treatment (designated BCLC5-C8.1 cells). *in vitro* transcribed neo-SGR RNA was then electroporated into the cured BCLC5-C8.1 cells and transduction efficiencies were determined 21 days post-G418 selection by counting the resulting colonies. Colony formation efficiency increased in BCLC5-C8.1 cells compared to their parental BCLC5 or Huh-7.5 cells (6- and 3-fold, respectively). Similarly, short-term replication efficiency was increased in BCLC5-C8.1 cells as deduced by the reporter activity of a SGR-carrying *Gaussia* luciferase (GLuc-SGR). Since miR122 has been shown to facilitate HCV replication, we assessed miR122 expression in the BCLC5 cell line and its derivatives by real time qRT-PCR. Neither BCLC5, BCLC5-C8, nor BCLC5-C8.1 cells expressed endogenous miR122. Therefore, we examined whether ectopic miR122 expression could enhance HCV replication in BCLC5-C8.1 cells. We then selected one clone that expressed miR122 at comparable levels to Huh-7.5 cells and analyzed HCV replication in these cells. BCLC-5 C8.1/mir122 cells exhibited a 3- to 4-fold increase in GLuc-SGR replication as deduced by reporter secretion and intracellular HCV RNA determination. Similar results were obtained upon electroporation of full-length HCV RNA. In conclusion, we have

established two new cell lines, BCLC5-C8.1 and BCLC5-C8.1/miR122, which are permissive for HCV replication. Our results reveal that miR122 is not essential for HCV replication; rather, it acts as an enhancer only in those cells with increased HCV replication tolerance.

Keywords: HCV, HCV replication, HCV cell culture.

P-157

Hepatitis C virus NS3/4A quasispecies diversity in acute hepatitis C infection in HIV-1 coinfected patients

María Nevot[1], Cristina Andrés*[1], Christoph Boesecke[2], Mariona Parera[1], Sandra Franco[1], Boris Rebollo[3], Cristina Tural[3], Bonaventura Clotet[1,3], Jürgen Rockstroh[2], Miguel Ángel Martínez[1]

(1) Fundació irsiCaixa. Hospital Germans Trias i Pujol. Badalona (2) Immunologische Ambulanz. Bonn University Hospital. Bonn, Germany (3) Fundació Lluita contra la Sida. Hospital Germans Trias i Pujol. Badalona.

In the last decade, acute hepatitis C (AHC) has increased among human immunodeficiency virus type 1 (HIV-1)-positive men who have sex with men (MSM). Thus, predictors of hepatitis C virus (HCV) clearance and epidemiological networks of viral transmission are of interest. We characterized the diversity and catalytic efficiency of protease HCV NS3/4A quasispecies in acute hepatitis C patients co-infected with HIV-1. The catalytic efficiency of the dominant quasispecies in plasma samples obtained from 18 HIV-positive MSM at the time of hepatitis diagnosis was assayed for mitochondrial antiviral signaling protein (MAVS) cleavage. Less nucleotide quasispecies diversity was detected compared to 26 HIV-1-infected men with chronic hepatitis C (CHC) genotype 1a ($P <$ 0001). Phylogenetic analysis identified two clusters of patients with highly related viruses, suggesting a common source of HCV infection. None of the patients spontaneously cleared HCV, though 78% of the treated patients achieved a sustained virologic response after early treatment with pegylated interferon (pegIFN) plus ribavirin (RBV). The synonymous-nonsynonymous (ds/dn) mutation ratio, a marker of selective pressure, was higher in AHC compared to CHC ($P < .0001$). NS3/4A proteases from AHC patients exhibited higher catalytic efficiency compared to CHC patients ($P < .0001$). Thus, the presence of epidemiological networks of HCV transmission was confirmed among HIV-1-positive MSM. Also, substantial genetic diversity was demonstrated in AHC. NS3/4A protease efficiency cleaving MAVS may be associated with the response to pegIFN/RBV treatment.

Keywords: HCV, quasispecies, protease, acute infection, HIV co-infection, catalytic efficiency.

P-158

Study of hepatitis C virus superinfection after liver transplantation by ultra-deep pyrosequencing

Josep Gregori Font*[1,2], Sofía Pérez del Pulgar[3], Patricia González[3], Damir García Cehic[1], Santseharay Ramirez[3,4], María Cubero León[1,2], Juan I. Esteban[1], Xavier Forns[3], Josep Quer[1]

(1) Laboratori Malalties Hepàtiques. VHIR, Hospital Universitari Vall d'Hebron. Barcelona (2) Business Development. Roche Diagnostics SL. Sant Cugat del Vallès. Barcelona (3) Servicio de Hepatología, Hospital Clinic. Hospital Clinic, IDIBAPS, CIBERehd. Barcelona (4) Copenhagen Hepatitis C Program, Department of Infectious Diseases and Clinical Research Centre. Copenhagen University Hospital. Denmark.

Backgroundand aims: Hepatitis C virus (HCV) chronic infection is the main indication for liver transplantation (LT). The limitation of organ supply has led to the use of grafts from extended-criteria donors, such as HCV-positive donors. In these particular cases, superinfection with two different viral populations occurs, leading to the dominance of one strain over the other. In a previous study, we examined early viral kinetics during superinfection by cloning and sequencing, using a limited number of clones (Ramirez S. et al., 2010). Thus, the aim of this study was to analyze HCV superinfection after LT by next-generation sequencing.

Methods: We included 6 HCV-infected patients who underwent LT with HCV-infected grafts. Serum samples were collected before (donor and recipient) and after LT (days 1 and 2, week 1, months 1, 4, 6 and 12) and analyzed by ultra-deep pyrosequencing (UDPS) using the 454 GS-FLX platform (Roche). Viral complexity was measured by mutation frequency and genetic diversity.

Results: Successive expansions and contractions of quasispecies were observed, evolving in all cases towards a more homogeneous population, with a relatively low genetic variability. In patients 1, 3 and 5, the donor population outcompeted the recipient virus immediately after LT (day1), whereas in cases 2, 4 and 6, the recipient virus overtook the donor's. In all cases, the most complex viral population excluded the other and became dominant. In cases 1, 2, 4 and 6, minority mutants derived from the donor or the recipient were detected at various points after LT regardless of the final result of the *in vivo* competition. Interestingly, in case 2, viral coexistence lasted even after the first year after LT.

Conclusions: Our results show that during superinfection with a different HCV strain in the LT, the viral population with the highest diversity always outcompetes the other and becomes dominant. The exclusion of non-dominant can take place as early as the first day or after several months following LT. However, the excluded virus may remain as a minority population (even after 1 year) and could emerge if there were any changes in the environment.

Keywords: HCV, multi-infection, liver transplant, NGS, UDPS, in-vivo competition, evolution.

P-159

Natural evolution of the hepatitis B virus quasispecies and oral antiviral treatment-induced changes analyzed by massive sequencing and evaluated by the entropy of the viral population

María Homs*[1,2], Josep Gregori[3,4], Josep Quer[1,3], David Tabernero[1,2], Silvia Camós[2], Rafael Esteban[1,3], María Buti[1,3], Francisco Rodríguez Frías[1,2]

(1) Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd). University Hospital Vall d'Hebron.Barcelona (2) Biochemistry Department. University Hospital Vall d'Hebron. Barcelona (3) Hepatology Department. University Hospital Vall d'Hebron. Barcelona(4) Statistics Department. Biology Faculty, Universitat de Barcelona.

Introduction. The high complexity of the Hepatitis B Virus (HBV) quasispecies was associated with HBeAg seroconversion and the likelihood of response to antiviral therapy. Shannon Entropy (SE) represents the viral complexity and can be obtained from massive sequencing data (UDPS). The precore / Core (PC / C) region is the least overlap within the HBV genome and thus the most appropriate to study variability.

Objective. To analyze the evolution of the quasispecies complexity in chronic hepatitis B (CHB) patients who received antiviral treatment, by comparing SE from samples collected prior to antiviral treatment and at the time of viral reactivation.

Methods. The pC / C region (nt 1757-2149) was UDPS-analyzed in 30 samples from 10 CHB patients (4 genotype A, 6 D, 8 HBeAg+ve, 2 HBeAg-ve). For each patient, three samples were included: one at the diagnosis of CHB, another during the follow-up prior to treatment (mean 19 months) and the third during lamivudine treatment, at the time of viral reactivation (mean 20 months). We defined a period of no treatment (NT = $SE_{PriorToTreatment}$-$SE_{Baseline}$) and a period of non-response to treatment (VBK = $SE_{Lamivudine}$-$SE_{PriorToTreatment}$).

Results. In 60% of cases there was an increase of the SE after NT and a decrease after VBK. In 20% of cases complexity was maintained during NT and decreased at VBK. In the remaining two cases the complexity decreased during NT but increased at VBK.

Conclusions. Predominantly, HBV quasispecies complexity increases during natural evolution probably due to the pressure of the host's immune system. In addition, the decrease of complexity after VBK seems to agree with the selection of variants resistant to antiviral treatment. Nevertheless, the observation of other dynamic patterns of complexity suggests the possible role of other factors.

Keywords: HBV quasispecies, ultra-deep pyrosequencing, Shannon entropy, precore / core region.

P-160

Dynamic complexity of hepatitis B virus quasispecies in a surface / polymerase overlapping region during treatment with nucleoside / nucleotide analog

David Tabernero*[1,2], Francisco Rodríguez-Frías[1,3], Rosario Casillas[4], Josep Gregori[4,5], Maria Homs[1,3], Josep Quer[1,4], Marta Mosquera[3], Sílvia Camós[3], Clara Ramírez[2], Rafael Esteban[1,4], Maria Buti[1,4]

(1) Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd). University Hospital Vall d'Hebron. Barcelona (2) Biochemistry. University Hospital Vall d'Hebron . Barcelona (3) Biochemistry Department. University Hospital Vall d'Hebron. Barcelona (4) Hepatology Department. University Hospital Vall d'Hebron. Barcelona (5) Statistics Department. Biology Faculty, Universitat de Barcelona.

Background and aims: Genetic variability of hepatitis B virus (HBV) in the reverse transcriptase (RT) region associated with nucleos(t)ide analogs (Nucs) can affect epitopes from surface (S) proteins. This study quantified changes in the S gene and RT region during lamivudine (LMV) treatment, using ultra-deep pyrosequencing (UDPS).

Methods: In serum samples from 9 Nuc-naïve chronic hepatitis B patients (5 HBeAg+ve, 5 genotype A and 4 D), codons s91-s200 from S gene and rt100-rt208 from RT region were UDPS analyzed (GS-FLX Titanium, Roche) before LMV (9 samples) and after viral breakthrough (VBK) (9 samples). Genetic variability of each sample was estimated by calculation of Shannon entropy (SE).

Results: 440993 sequences were analyzed. SE was not significantly different between HBeAg+ve and HBeAg-ve, or genotype A and D, at baseline or after VBK. However, SE increased (greater variability) during treatment in 80% of HBeAg+ve and decreased in 75% of HBeAg-ve. In the major hydrophilic region (MHR) of S gene, variability was mainly observed in polymorphic positions with no known association with immune response escape. In 3 cases, sP120T variant, which is associated with immune escape and the compensatory variant rtT128N in RT, was detected. The "a" determinant showed a high degree of conservation: 33% of patients without changes, the remainder with polymorphic changes or changes previously unassociated with immune escape. Outside MHR, greatest variability was seen in positions directly associated with RT mutations implicated in LMV resistance (sE164D/rtV173L, sI195M/rtM204V, sW196L/rtM204I and sM198I/rtV207I). In RT region, in addition to changes expected after LMV-VBK in domains B and C, rtA200V variant was observed in 22% and rtT184S (associated with entecavir resistance) in 3.7% of cases. The rtV163I variant was selected or increased during treatment.

Conclusions: HBV genetic variability follows different trends during Nuc therapy according to HBeAg status, which could be related to the different response of HBeAg+ve and HBeAg-ve patients to these treatments. The prevalence of escape mutations in the "a" determinant is very low both before and after Nuc treatment, which mainly affects positions outside this epitope. Study

of additional patients will help confirm these results.

Study funded by the FIS project PI11/01973.

Keywords: HBV genetic variability, quasispecies, ultra-deep pyrosequencing, Shannon entropy, surface/reverse transcriptase overlap, immune escape, Nucs resistance.

P-161

Molecular epidemiology of hepatitis A virus in Spain

Silvia Calleja*[1], Alejandro González Praetorius[1,3], José Manuel Echevarrría[2], Ana Avellón[1]

[1] Unidad de Hepatitis. Centro Nacional de Microbiología.Majadahonda, Madrid [2] Área de Virología. Centro Nacional de Microbiología. Majdahonda, Madrid. [3] Departamento de Microbiología. Hospital Universitario de Guadalajara.

Introduction and Objective: Human hepatitis A virus (HAV), causes near 1,4 millions of infections worldwide each year (WHO). HAV outbreaks are mainly fecal-oral route waterborne and foodborne related, while in approximately 40% of the reported cases of hepatitis A the source of infection cannot be identified.HAV RNA encodes four capsid proteins, (VP) 1-4 and can be classified into 6 genotypes (G): I, II, and III (humans), IV, V, and VI (simians). G I to III are each further classified into subtypes A and B. G-I is the most common, worldwide distributed being G-IA its more common subtype**.** In Spainfew studies have been made restricted to Valencia, Catalonia and Galicia involving mostly outbreaks due to G-IB. The objective of this work is presenting HAV molecular epidemiology data from different regions of Spain detected along at least 10 years.

Materials and methods: 5 overlapping two-step PCRs were designed for amplifying VPs and analysis of sequences was performed individually in each VP. Regarding VP2 analysis (about 511 nt length), nucleotide HAV sequences were compared to 62 previously reported HAV sequences retrieved from GenBank (26 G-IA, 14 G-IB, 6 G-IIA, 1 G-IIB, 13G-IIIA, 2G-IIIB). MEGA 5.1 software was used for alignment and phylogenetic tree construction (neighbor joining method, 1000 bootstrap re-sampling test). HAV positive RNA extracts coming from samples received in the National Center of Microbiology till 2012 were included.

Results and conclusions: complete VP2 analysis was able to separate G clusters with the following bootstrap values (BV) of the branches: G-I (BV=94%), G-II (BV=100%), G-III (BV=100%). Furthermore it differentiates sub-genotypes: G-IA (BV=92%), G-IB (BV=99%), G-IIA (BV=99%), G-IIIA (BV=99%) and G-IIIB (BV=100%). Preliminary results of 18 complete VP2 sequences indicate that in Spain G-IA is the most frequent detected but G-IB also circulates. Additionally our analysis shows its ability of clustering sequences (BV: 100%) associated to outbreaks both in G-IA and G-IB. The VP2 analysis was able not only to precisely discriminate all genotypes and geno-subtypes, but also allows the identification of outbreak clusters. The compar-

ative analysis of the remaining VPs will reveal us the ability of each, for phylogenetic analysis. Although preliminary results indicate the G-I predominance, the inclusion of more samples will let us having a wider idea of HAV molecular epidemiology in Spain.

Keywords: HAV, molecular epidemiology.

P-162

Characterization of E2 N -terminal glycoprotein of the hepatitis C virus genotype 1b

Rocío Esteban*[1], María del Carmen García Galera[1], José Manuel Echevarría[2], Ana Avellón[1]

[1] Unidad de Hepatitis. Centro Nacional de Microbiología. Majadhonda, Madrid [2] Área de Virología. Centro Nacional de Microbiología. Majadhonda, Madrid.

Introduction and Objective: Despite of highly effective new therapy, dynamics of Hepatitis C virus (HCV) infection is still unknown in many aspects. HCV great diversity envelope glycoproteins (E1-E2) play an important role in entry to hepatocytes, neutralizing antibody (nAb) binding and disease outcome (Edwards 2012). E2 includes two hypervariable regions named HVR1 (aa384-410) and HVR2 (aa471-482). E2 N-terminus is also involved in SRB1 interaction (blocked by Ab against aa396-407) and HCV CD81 binding (blocked by Ab against aa412-423 [linear epitope] and residues forming conformational epitope). Moreover HVR1 is hypothesized to function as an immunological decoy, due to its high ability of mutational escape being involved in the disease outcome to chronicity. The objective of the present study is to design a system for characterize the HCV genotype 1b N-terminal domain of E2 glycoprotein (HVR1 and HVR2) to study the sequence in several HCV-1b RNA extracts determining sequence variability and focusing in nAb amino acid targets.

Methods: A RT nested PCR assay HCV-1b specific to amplify 648 nt including E2 N-terminus was designed. Oligonucleotide sequences were determined analyzing a total of 150 GenBank HCV-1b sequences.

Results and Conclusions: Preliminary results of 19 sequences were as follows. The variability considering the average variability percentage in each position was of 34.5% and 24.1% in HVR1 and HVR2 respectively. The 100% conserved positions were T385, G389, G406, Q409 [in HVR1] and P471, I472, D481 [in HVR2]. Regarding main linear epitopes, the aa396-407 targeting SRB1 nAb had a variability of 29.8%. On the other hand, the linear HCV CD81 binding site in aa412-423 had a 6.1% of variability with 9 of its 12 residues 100% conserved. In the case of the conformational epitopes, the five more critical residues for E2 binding were W420, Y527, W529, G530 and D535, being all 100% conserved. Additionally, two of the studied samples include an in-phase insertion between positions aa383-384 and aa445-446 respectively while one sample contained an in-phase deletion (aa388). Although pre-

liminary, these results evidence the high degree of variability of such of the studied regions, specially the related to SRB1. On the contrary those regions associated to the CD81 binding were apparently more conserved. With the analysis of these 19 and additional samples a consensus sequence should be defined to be used in future for mutation pattern comparison.

Keywords: HCV, HVR.

P-163

Molecular epidemiology of hepatitis E virus genotype 3 in Spain

Ana Avellon*[1], Marta Fogeda[1], Lucía Morago[1], José Manuel Echevarría[2]

[1] Unidad de Hepatitis. Centro Nacional de Microbiología. Majadhonda, Madrid [2] Área de Virología. Centro Nacional de Microbiología Majadhonda, Madrid .

Introduction and Objective: human hepatitis E virus (HEV) genotype (G) 3 is causing acute hepatitis in Europe, also in Spain (Echevarría 2011) and has been detected in pork and wild boar samples (G-3e, G-3f), and in sewage from Barcelona (G-3). Although the exact mechanism operating virus transmission to humans remains unclear, HEV is known to be water- and food-borne related (the meat of the infected animals is the main suspicious source for infections). G-3 produces sporadic acute hepatitis and its diagnosis depends on the severity of the symptoms, which may be underestimating the incidence. Since the year 2007, the National Centre of Microbiology is receiving an increasing number of samples for HEV diagnosis, and has collected about 40 HEV RNA positive samples until 2012. The objective of the present study is to analyze the genomes found in samples containing G-3 HEV strains in order to identify the sub-genotype and to investigate the genetic relationships existing among them, and also to other G-3 strains reported from Spain.

Materials and methods: a RT nested PCR system amplifying 482nt (ORF2), including capsid proteins was designed. The preliminary analysis was performed with 12 amplified sequences being compared to at least 75 previously reported HEV sequences retrieved from GenBank (1 G-I, 1 G-II, 1 G-IV, and 72 G-III). MEGA 5.1 software was used for alignment and phylogenetic tree construction (neighbor joining method, 1000 bootstrap (BV) resampling test). Two analysis were performed, first over a 170nt fragment, comparing samples to all 75 GenBank sequences and second over a 453nt fragment, comparing samples with a total of 28 sequences.

Results and conclusions: most of the sequences (n=10) corresponded to G-3f (BV 82/67), clustering in a big group including sequences obtained from pork meat in marketed Spanish products. One sequence grouped as G-3c (BV 97/67) in a cluster including one sequence obtained from wild boar in Germany. Finally, one other sequence clustered in a group (BV 100/99) with two sequences coming from pork meat in marketed Spanish products, not grouping with any G-3 subgenotype. Although preliminary, these results

show that in Spain, a variety of sub-genotypes might be circulating, including one for which it seems not to be available sequences for comparison. It is interesting to go in depth in the comparative study of source and clinical cases sequences, including more of them and analyzing bigger fragments.

Keywords: HEV, molecular epidemiology.

P-164

Structural studies of the head-subdomain of the human CD81 large extra-cellular loop

Pietro Roversi*[1], Marina Ondiviela[2], Nicola G. Abrescia[2]

(1) Lab 2 Microscopia. CIC Biogune. Derio, Vizcaya
(2) Structural Biology Unit. CIC BIOGUNE. Derio Vizcaya.

The human tetraspanin hCD81 is one of the cellular receptors that Hepatitis C virus (HCV) uses to gain entry into hepatocytes. A few years ago, theo 5-helical bundle structure of its long extra-cellular loop (LEL) was elucidated by X-ray crystallography (PDBIDs 1G8Q and 1IV5, [1],[2]). Recently, an NMR study has suggested unstructured elements in the hCD81-LEL head-subdomain involved in HCV attachment [3].

Here, we report three new crystal structures of hCD81-LEL, bringing the total number of crystallographically independent molecules to twelve. Exhaustive comparative structural analysis over this ensemble of molecules details the high structural dynamism (pairwise C_{alpha} rmsd: 0.4Å <= C_{alpha} rmsd <= 5Å) of the CD81-LEL head-subdomain, providing atomic information on the recognition module of HCV.

These results are central for structure-based drug design of inhibitors of HCV attachment.

[1] Kitadokoro K et al, EMBO J. 2001 Jan 15;20(1-2):12-8
[2] Kitadokoro K et al, Biol Chem. 2002 Sep;383(9):1447-52
[3] Rajesh S et al, J Virol 2012 Sep;86(18):9606-16

Keywords: CD81-LEL, HCV, X-ray crystallography.

P-165

Amplification systems for the characterization of hepatitis C virus NS3 protease

María del Carmen García Galera*[1], José Manuel Echevarría[2], Ana Avellón[1]

[1] Unidad de Hepatitis. Centro Nacional de Microbiología. Majadhonda, Madrid [2] Área de Virología. Centro Nacional de Microbiología. Majadhonda, Madrid.

Introduction and Objective: Hepatitis C virus (HCV) standard therapy includes nowadays antiviral compounds targeting NS3 protease (NS3-P) which activity is essential for polyprotein cleavage, so called protease inhibitors (PI). NS3-P is considered moderately conserved among genotypes and in its amino acid sequence, several substitutions of residues have been associated to inefficacy of PI. The rapid emergence of PI resistant variants has been demonstrated when they are used as single therapy, nevertheless the current treatment algorithms are reaching the objective of restrain resistance variants. However, characterizing resistance should be available in reference laboratories for its use in clinical trials or even the characterization of individual patients with therapeutic failure. The objective of this study is the design and standardization of systems for the complete NS3-P amplification and amino acid analysis.

Materials and methods: RT nested PCR systems specific for genotype (G) 1a, 1b and 3 amplifying the total NS3-P (about 850 nt) were designed. Amplification and analysis was initially performed in 38 samples corresponding to non PI previously treated patients, 16 harboring G-1b, 1 G-1a and 21 G-3.

Results and conclusions: the three PCR systems were proved to amplify each genotype providing about 700 nt of proper sequence for analysis. Eight major positions involved in resistance development to PIs were analyzed with the following results: wild type (WT) amino acid according to Cento V. et al 2012 consensus in positions aa36, 54, 55, 80, 155, 156 and 168. On the other hand, aa170 in G-1b presented I170 (38%) and V170, considered WT (62%). Additionally other minor or secondary positions were 100% WT aa41, 43, 109, 138 and 158 while aa175 presented L175 in G-1b. Although preliminary, these results show, as expected, a few rate of mutations in PI naïve patients; however we have found aa170 variability according to the recently published consensus sequence. The inclusion of additional samples will help to define the consensus sequence for comparison.

Keywords: HCV, viral resistance, protease.

P-166

Evaluación de secuenciación sanger, clonación, y ultrasecuenciación (454-ROCHE) para la detección y vigilancia de mutaciones de resistencia a inhibidores de la proteasa y polimerasa del virus de la hepatitis C (VHC)

Karina Salvatierra[1], Elisa Martró[2], Alejandro Artacho[1], Marina Berenguer[3], F. Xavier López Labrador*[1]

(1) Lab. Virología, Area de Genómica i Salut. Centro Superior de Salud Pública (CSISP). Valencia (2) Servicio de Microbiología. Hospital Universitari Germans Trias i Pujol. Badalona (3) Servicio de Medicina Digestiva. Hospital Universitario La Fe. Valencia.

Objetivos. Se han caracterizado mutaciones de resistencia para varios nuevos antivirales específicos contra el VHC (STAT-C). El impacto de estas mutaciones se está explorando por métodos de secuenciación convencionales y de ultrasecuenciación, que comparamos en este trabajo.

Métodos. Se secuenció la totalidad de las regiones NS3/4A proteasa (181aa) y la NS5B polimerasa (590aa) del VHC por química Sanger en aislamientos de 30 pacientes con infección crónica por VHC subtipo 1b. En diez pacientes seleccionados, los productos de PCR se clonaron, y se secuenciaron >20 clones moleculares para cada región. Además, en cinco de estos pacientes, la proteasa HCV-NS3/4A también se evaluaó por una estrategia alternativa de ultrasecuenciación utilizando códigos de barras multiplexados en un equipo 454-Roche-FLX+. Se compararon las mutaciones detectadas mediante Sanger con las obtenidas a partir de la clonación, o de la ultrasecuenciación.

Resultados. Mediante secuenciación convencionales Sanger se detectaron variaciones / polimorfismos entre pacientes en varios sitios de la proteasa NS3 y / o polimerasa NS5B, algunos asociados con resistencias (en su mayoría a inhibidores no nucleosídicos, NNI). No se detectaron mutaciones primarias de resistencia a Boceprevir o Telaprevir o (inhibidores de la proteasa NS3) o inhibidores nucleosídicos (NI) de la polimerasa NS5B ni por secuenciación Sanger, ni por clonación y secuenciación. Sin embargo, algunas resistencias a inhibidores no-nucleosídicos (NNI) de NS5B no detectadas por Sanger, fueron detectadas por clonación en varios casos. La ultrasecuenciación (NS3 proteasa secuencias por paciente: 3.600-36.000; cobertura promedio 200x-3.500x) permitió la identificación de muchas más variantes, incluyendo casi todas las posiciones implicadas en resistencias a inhibidores de la proteasa, en algún sitios a tasas de <10% de la población viral.

Conclusiones. La secuenciación convencional Sanger puede detectar variaciones / polimorfismos entre pacientes en sitios asociados con resistencias (principalmente a NNI). El análisis por clonación es más sensible para la detección de mutaciones dentro de cada paciente, pero es muy laborioso. Una alternativa basada en ultrasecuenciación 454 con muestras multiplexadas permite un análisis más rápido y sensible de mutaciones de resistencia a la proteasa NS3 del VHC. En re-

sumen, estas técnicas pueden ser útiles para la evaluación y la vigilancia de las resistencias del VHC a nuevos antivirales STAT-C.

Keywords: VHC, resistencias, proteasa, polimerasas, ultrasecuenciación.

P-167

HCV cell-to-cell transmission: differential role of apolipoproteins B and E

Francisa M. Jiménez[1], Virginia M. Gondar*[1], Ignacio B. Español[1] George K. Koutsoudakis[2], Manuel L. Cabrera[3], Pedro L. Majano[1]

(1) Unidad de Biología Molecular. Instituto de Investigación Biomédica Hospital La Princesa. Madrid
(2) Unidad de Hígado. Hospital Clínic de Barcelona.
(3) Departmento de Biología Celular e Inmunología. Centro de Biología Molecular Severo Ochoa. Madrid.

Hepatitis C virus (HCV) infects primarily hepatocytes by two distinct routes: cell-free particle transmission using sinusoidal blood as mode of propagation and engaging specific cellular receptors, and cell-to-cell transmission by which virions are transferred directly from an infected to an adjacent uninfected cell mediated by mechanisms not well defined yet. As it has been proposed that HCV egress occurs in association with the very low-density lipoproteins (VLDL) secretory pathway, and that apolipoprotein B (apoB) and E (apoE) are necessary for HCV assembly, we decided to study if these VLDL components affect HCV cell-to-cell transmission *in vitro*.

We assessed the effect of apoB and apoE depletion in a HCV cell-to-cell transmission assay by means of infection foci size where cell-free infection was inhibited by an agarose overlay or the addition of cell-free HCV blocking antibody.Our results demonstrate that apoE, but not apoB, was determinant for HCV cell-to-cell spreading. Also, apoB knockdown did not impair the intracellular association between apoE and HCV RNA.

This study shows that HCV cell-to-cell transmission is apoB independent and therefore suggests that cell-to-cell and cell-free HCV infections are differentially regulated. The identification of specific host factors involved in these routes of transmission may help to develop new therapeutic targets and advance our understanding of HCV pathogenesis.

Keywords: HCV, apolipoproteins, VLDL, cell-to-cell, transmission.

P-168

Isolation and characterization of a West Nile virus mutant with increased resistance to acidotropic compounds

Miguel A. Martín Acebes*[1], Ana Belén Blázquez[1], Nereida Jiménez de Oya[1], Estela Escribano Romero[1], Pei-Yong Shi[2], Juan Carlos Sáiz[1]

(1) Department of Biotecnology. INIA. Madrid (2) Wadsworth Center. New York State Department of Health. Albany, USA.

West Nile virus (WNV) is a worldwide distributed mosquito-borne flavivirus that naturally cycles between birds and mosquitoes, although it can infect multiple vertebrate hosts including horses and humans. This virus is responsible for recurrent epidemics of febrile illness and encephalitis, and has recently become a global concern. As the other flaviviruses, WNV requires to transit through intracellular acidic compartments at two different steps to complete its infectious cycle. These include the endosomes involved on membrane fusion during viral entry, and the *trans*-Golgi network involved on virus maturation. In this study, we followed a genetic approach to study the connections between viral components andacidic pH. A WNV mutant with increased resistance to the acidotropic compound NH_4Cl, which blocks organelle acidification and inhibits WNV infection, was selected. Nucleotide sequencing revealed that this mutant displayed a single amino acid substitution (Lys 3 to Glu) on the highly basic internal capsid or core (C) protein. The functional role of this replacement was confirmed by its introduction into a WNV infectious clone. The mechanism of escape of the mutant from the action of the acidotropic compound NH_4Cl was based on an increase on infectious particle production but not on genome replication. This single amino acid substitution also increased resistance to other inhibitor of the acidification, concanamycin A, and induced a severe reduction of the neurovirulence in mice. The findings here reported unveil a non-previously assessed connection between the C viral protein and the acidic pH necessary for entry and proper exit of flaviviruses. These results also identify the mutated position within the N-terminus of the C protein as an important determinant of the virulence of WNV.

Keywords: West Nile virus (WNV), flavivirus, acidic pH, mutant.

P-169

Study on the involvement of the cellular escrt machinery in vaccinia virus infection

Maria M. Lorenzo[1], Ana Cáceres[2], Juana M. Sánchez Puig[1], Mariano Esteban[2], Alberto Fraile Ramos[3], Rafael Blasco*[1]

(1) Departamento de Biotecnología. INIA.Madrid (2) Biología Molecular y Celular. Centro Nacional de Biotecnología - CSIC. Cantoblanco, Madrid (3) Departamento de Biología Celular - Facultad de Medicina. Universidad Complutense de Madrid.

The endosomal sorting complex required for transport (ESCRT) machinery controls the incorporation of cargo into intraluminal vesicles of multivesicular bodies. ESCRT is used during envelopment of many RNA viruses and trafficking of viral proteins of some DNA viruses. Other viruses mature independently of ESCRT components, relying on the intrinsic behavior of their viral matrix and envelope proteins. Vps4 protein is an ATPase required to dissociate ESCRT complexes from the membrane after vesicle release. The two human proteins Vps4-A and Vps4-B form heteromeric complexes, that associate with endosomal compartments and multivesicular bodies. Mutant human Vps4-A (E228Q) and Vps4-B (E235Q) proteins, harbouring single amino acid changes in their ATPAse domain, display a dominant-negative phenotype. In addition to a role in virus scission from cellular membranes, recent publications suggest that ESCRT is required for the entry of some viruses, like Old World arenavirus, LASV and LCMV, that invade the host cell via endosomal compartments.

To analyze if a functional ESCRT system is required for Vaccinia infection, we have used different experimental approaches: 1) siRNAs were used to inhibit the expression of Vps4 in the cells prior to infection, 2) dominant negative Vps4-GFP was expressed prior to infection, to allow monitoring of the infection process in transfected cells and 3) Vps4b, or the dominant negative version, was expressed in vaccinia virus recombinants under viral early/late promoters. Our results indicate that inhibition of Vps4 prior to infection reduces virus production only moderately, whereas expression of dominant negative Vps4 during the infection did not affect significantly virus transmission. Those observations indicate that the ESCRT machinery is not required in the vaccinia virus exit from the cell.

Keywords: virus exit, virus transmission, viral envelope, ESCRT.

P-170

Synergistic activity profile of carbosilane dendrimer G2-STE16 in combination with different dendrimers and antiretrovirals as topical microbicide against HIV-1

Daniel Sepúlveda Crespo*[1], Raquel Lorente[1], Javier Sánchez Nieves[2], Rafael Gómez[2], Francisco J. de la Mata[2], José L. Jiménez[3], María Ángeles Muñoz Fernández[1-3]

(1) Laboratorio de Inmunobiología Molecular. Hospital General Universitario Gregorio Marañón. Madrid (2) Departamento de Química Inorgánica. Universidad de Alcalá de Henares. Madrid (3) Plataforma de Laboratorio. Hospital General Universitario Gregorio Marañón. Madrid.

Background. Self-administered oral pre-exposure prophylaxis or topical microbicides may be very helpful tools for women and homosexuals to decrease new HIV infections. Polyanionic carbosilane dendrimers are safety and effective compounds against HIV with great potential as topical micro-

bicides. Antiretrovirals (ARV) are the most advanced microbicides. A combination of at least two different families of ARV is more effective than monotherapy in the treatment of HIV infection. The combination approach should be taken into consideration when designing new microbicide.

Objective. To research the G2-STE16 carbosilane dendrimer synergistic activity profiles in combination with other carbosilane dendrimers or ARV as topical microbicide against HIV-1.

Methods. TZM-bl cell line was treated with G2-STE16, G2-S24P, G2S16, tenofovir (TFV) and maraviroc (MRV), alone or in different combinations. After 1h were infected with X4-HIV-1_{NL4-3}, R5-HIV-1_{NLAD8} or X4/R5-HIV-$1_{89.6}$. After 48h of infection, the 50% effective concentration (EC_{50}) was determined from luciferase activity. Analysis of combined effects was performed using CalcuSyn software.

Results. We have shown that G2-STE16 had a potent activity against X4-HIV-1_{NL4-3}, R5-HIV-1_{NLAD8} or X4/R5-HIV-$1_{89.6}$ in TZM-bl. From the two-drug combinations, combination indices weighted average (CI_{wt}) has shown synergistic activity profile between G2-STE16/G2-S24P, G2-STE16/G2-S16, G2-STE16/TFV and G2-STE16/MRV. Moreover, these combinations did not affect to PBMC and $CD4^+$ T-cells proliferation, inflammation, CD4 receptor and CCR5 and CXCR4 co-receptors expression.

Conclusions. We have demonstrated that the combination of G2-STE16 with other dendrimers or ARV has increased the antiviral potency of each individual drug. Our results support further researches on dendrimer/dendrimer or dendrimer/ARV as topical microbicide against HIV-1.

Keywords: microbicide, dendrimer, antiretroviral, HIV, synergy.

P-171

Understanding the role of histo-blood group antigens (HBGAS) in norovirus-host interactions

Noelia Carmona Vicente[1], Manuel Fernández Jiménez[1], Jesús Rodríguez Díaz[1], Carlos J Téllez Castillo[1], Javier Buesa*[1,2]

[1] Department de Microbiología. Facultat de Medicina, Universitat de València. [2] Servicio de Microbiología Clínica. Hospital Clínico Universitario de Valencia.

Background and objectives. Noroviruses (NoVs), members of the *Caliciviridae* family, are small, positive-polarity RNA viruses and the most important cause of human foodborne viral gastroenteritis worldwide. They are classified in 5 genogroups, although genogroup I (GI) and GII cause most human norovirus infections. Each genogroup is subdivided into 9 and 21 different genotypes, respectively (Lindesmith et al., 2013). Different genotype GII.4 variants have emerged and caused the majority of norovirus outbreaks and sporadic infections over the past 2 decades. NoVs recognize human histo-blood group antigens (HBGAs) as receptors, a relationship that has led to a major breakthrough in NoV research. Our aim was to investigate the binding of different

NoV VLPs to saliva samples from patients who suffered NoV infection.

Materials and methods. The secretor (FUT2+) and non-secretor (FUT2-) status was investigated in 22 convalescent patients of NoV infection. DNA was extracted from saliva samples and the FUT2 gene was analysed by PCR and *Ava*II restriction. The ABO and Lewis antigens were phenotyped by ELISA with monoclonal antibodies. Binding to saliva and blocking assays were conducted with NoV virus-like particles (VLPs) representative of GI.1 and GII.4 genotypes (GII.4-1999(v0), GII.4-2004(v2) and GII.4-2006b).

Results. By FUT2 genotyping and ABO/Lewis phenotyping assays 3 non-secretor, 5 homozigote secretor, and 14 heterozigote secretor individuals were identified, with different patterns of Lewis antigens: Le $^{a+b+}$ /Le $^{x-y+}$(76.5%), Le $^{a+b-}$ /Le $^{x+y-}$ (17.6%) y Le $^{a+b+}$ /Le $^{x+y+}$(5.9%). Binding assays with the different VLPs showed diverse patterns of attachment to the saliva samples. GI.1 VLPs strongly bound to A+ antigen and to type O saliva, but did not bind to B+ antigen and nonsecretor saliva, as previously reported. The GII.4-v0 and GII.4-v2 VLPs showed a very similar binding pattern, whereas GII.4-2006b VLPs bound stronger to B+ saliva. Monoclonal antibodies towards Le^b and Le^y significantly blocked the binding of the GII.4 variants VLPs to secretor but not to nonsecretor saliva samples. This blocking function was also observed with convalescent serum samples.

Conclusions. Different patterns of the interaction of NoV GI.1 and GII.4 variants with saliva samples are clearly dependent on the HBGAs saliva phenotypes. Blockade of the binding can be achieved with anti-Le mAbs and polyclonal convalescent sera.

Keywords: norovirus, genotypes, histo-blood group antigens, binding, saliva.

P-172

Late endosome-dependence for African swine fever virus entry

Covadonga Alonso*[1], Miguel Ángel Cuesta Geijo[1], Inmaculada Galindo[1], Bruno Hernáez[1], Raquel Muñoz Moreno[1]

[1] Departamento de Biotecnología. Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) Madrid.

The entry of double stranded DNA viruses is a complex process involving molecular signalling cascades triggered by virus-receptor binding, and a key determinant of tropism and pathogenesis. African swine fever virus (ASFV) endocytosis seems to be the crucial step that determines whether a productive infection takes place or not. ASFV enters the target cell by clathrin- and dynamin-dependent endocytosis, but also macropinocytosis could play a role in entry. Our data could shed light into this intriguing process. We found that ASFV infectivity was severely decreased by drugs that block endosomal acidification. In fact, both endocytosis and acidification were required for a successful ASFV infection. Signaling required for endosomal maturation palus a central role for infection especifically, small GT-

Pase Rab7 and phosphoinositide (PI) interconversion mediated by enzymes PI3K and PIKfyve.

All these data point out a determinant role for late endosomal compartments, the multivesicular bodies (MVBs) ans the late endosomes (LEs), both characterized by acid luminal pH. We found colocalization of capsid p72-labeled virions and an early endosome marker at 1-15 min postinfection, but not with further acidic compartments. Instead, viral core protein p150 colocalized with CD63 positive MBVs and Rab7 positive LEs. With adidification inhibitors, virions were retained intact inside endosomes and infection progression was blocked. These results imply that ASFV belongs to the category of late-penetrating viruses. The implications for the development of antivirals against early targets of ASFV will be discussed.

Keywords: virus entry, endocytosis, late endosome, multivesicular bodies, PI3K, PIKfyve, clathrin, dynamin, Rab GTPases, Rab7.

P-173

Cytotoxicity and capsid assembly of minute virus of mice in cerebellar mouse stem cells: implication in parvovirus neuropathogenesis

Jon Gil Ranedo[1], José M. Almendral del Río*[1,2]

(1) Virology and microbiology. Centro de Biología Molecular Severo Ochoa. Madrid .(2) Departamento de Biología Molecular. Universidad Autónoma de Madrid.

We described in the past that the "i" strain of the parvovirus minute virus of mice (MVMi) provokes a severe lost in cell density of the internal granular layer of the developing mouse cerebellum, halting the migration of neuroblasts to the external layer (Ramirez et al., J. Virol., 70: 8109, 1996). That neonatal infection caused evident neuropathological symptoms and motor dysfunctions. We have now examined the capacity of MVMi to infect mouse cerebellar stem cells (CbSC) in culture. We show that CbSC freshly explanted and cultured in selective conditions form neurospheres with active proliferative activity characterized by the expression of neural stem cell markers. The CbSC behaved fully permissive to MVMi, as the neurospheres were disrupted by viral infection at 2 dpi, and cells appeared with evident cpe. Importantly, the expression of the NS1 protein in the nucleus, and capsid formation, was demonstrated in most CbSCs. We conclude that CbSc affectation by MVMi may significantly underly the neuropathogenesis of this virus in newborn mice.

Keywords: parvovirus, cerebellum development, stem cell, capsid assembly.

P-174

Engineering the capsid of parvovirus minute virus of mice virions with heterologous peptides: effects on assembly and infectivity

José M. Almendral del Río[1,2]

(1) Biología Molecular. Universidad Autónoma de Madrid. (2) Department of Virology and Microbiology. Centro de Biología Molecular Severo Ochoa (CSIC-UAM) cantoblanco, Madrid.

Some members of the *Parvoviridae*, including the minute virus of mice (MVM), show oncolytic capacity against human cancer cells in culture and in animal experimental models. In order to explore whether the capsid of infectious MVM could be engineered to carry heterologous peptides increasing oncospecificity, several heterologous peptides binding vascular endothelial growth receptor (VEGF-R) were inserted at the spike of the capsid. The MVM-VEGFR mutants were viable to some extent, but show limited capacity to progress in culture of human transformed cells. Preliminar evidences suggest that the chimeric viruses harbor restrictions to maintain an stable functional capsid. We are currently characterizing the steric disturbance created by the inserted peptides on MVM capsid. Major findings and implications will be discussed.

Keywords: parvovirus, capsid engineering, assembly, infectivity, heterologous peptides.

P-175

The PDZ binding motifs of severe acute respiratory syndrome envelope protein are novel determinants of the viral pathogenesis

José M. Jiménez Guardeño*[1], Marta L. DeDiego[1], José L. Nieto-Torres[1], José A Regla-Nava[1], Raúl Fernández Delgado[1], Luis Enjuanes[1]

(1) Department of Molecular and Cell Biology. Centro Nacional de Biotecnología (CNB-CSIC) Madrid.

Severe acute respiratory syndrome coronavirus (SARS-CoV) appeared in China in 2002, infecting 8000 people worldwide and causing an average mortality of 10%. Previously, we demonstrated that a SARS-CoV lacking envelope (E) gene (SARS-CoV-deltaE) was attenuated in three different animal models. E gene codifies a small multifunctional protein, which contains three potential PDZ binding motifs (PBM). PBMs are recognized by PDZ domains, participating in protein-protein interaction processes relevant for virus life cycle, such as cell junctions, cellular polarity and signal transduction pathways. In the human proteome, more than 900 PDZ domains have been identified. To analyze the role of E protein PBMs in virus pathogenesis, two recombinant mouse adapted SARS-CoVs were generated: SARS-CoV-E-PBM1, which conserved one out of the three PBMs and SARS-CoV-E-deltaPBM, that lacked all PBMs. Deletion of either two or the three PBMs did not compromise virus growth in cell culture, as compared with a wild type virus (SARS-CoV-wt). To evaluate the relevance of E protein PBMs in virus

virulence, BALB/c mice were infected with mutant viruses. Mice infected with recombinant viruses containing at least one of E protein PBMs rapidly lost weight and died. In contrast, mice infected with SARS-CoV-deltaE and SARS-CoV-E-deltaPBM, which lacked all PBMs, survived and did not lose weight. The virus lacking the three E protein PBMs showed less lung damage, a reduction in the expression of proinflammatory cytokines, and decreased virus titers in lungs, as compared with the viruses containing at least one PBM. These results indicated that elimination of all E protein PBMs led to virus attenuation. Surprisingly, the virus containing just one PBM, caused similar pathology than the wild type virus. This data indicated that the presence of only one E protein PBM was sufficient to maintain virulence, possibly by interacting with cellular proteins containing PDZ domains. A new set of recombinant viruses is being generated to evaluate the relevance of each PBM in virus pathogenesis.

Keywords: SARS-CoV, virulence, envelope protein.

P-176

Integration of polydnaviruses DNA into the lepidopteran host genome

Laila Gasmi*[1], Agata.K Jakubowska[1], Juan Ferré Manzanero[1], Salvador Herrero Sendra[1]

[1] Department of Genetics. Universitat de Valencia.

Polydnaviruses (PDVs) are insect viruses characterized by polydisperse genomes organized as series of different circular DNAs. They are symbiotic viruses of endoparasitoid wasps belonging to the families *Braconidae* and *Ichneumonidae*. PDVs DNA are integrated into the parasitoid wasp genome and benefit from the wasp machinery for replication. They are injected together with the wasp eggs into the lepidopteran host in order to disrupt the insect immunity and development and to increase the success of the wasp parasitism. There are many studies showing that PDVs can infect directly lepidopteran cells and integrate into the cell DNA. But so far, no evidence of germ line integration in the lepidopteran host genome has been shown.

Transcriptome of the beet armyworm *Spodoptera exigua* revealed the presence of a number of unigenes sharing high homologies with PDV genes. In an effort to prove that PDV segments might be integrated into the lepidopteran genome during their evolutionary process, we determined and analyzed in detail the genomic sequences of two of these genes: one expressing a protein with unknown function, and the other expressing a protein with a lectin like domain. Both proteins are mainly expressed in the larval hemocytes and share high homologies with proteins of *Cotesia sp* bracoviruses. We have used genome walking in order to determine the whole PDV homolog sequences and we have determined the 3' and 5' boundaries of the studied genes. Analysis of the obtained sequences showed, in both cases, the presence of a bracovirus homolog sequence flanked by the lepidopteran genome. Analysis in detail of these sequences pointed out to the presence of potential transposable elements that

might have been involved in the integration of the viral sequence into the lepidopterangenome.

Sequence analyses suggest that some polydnavirus genes were integrated and maintained intothe lepidopteran host genome during the evolutionary process. This finding leads us to wonder about the possible evolutionary significance of these integrations and the possible role of the expressed proteins in the parasitism process.

Keywords: polydnavirus; *Spodoptera* exigua; germ line integration.

P-177

Henrietta Lacks' derived STINGs present a differential ability to induce IFN-ß

Estefanía Rodríguez García*[1], Estanislao Nistal Villán[1], Roberto Ferrero Laborda[1], Gloria González Aseguinolaza[1]

(1) Departamento de Terapia Génica y Hepatología. Fundación para la Investigación Médica Aplicada. Pamplona.

The innate immune system provides a primary line of defense against pathogens in eukaryotes. This defense system is based on the detection of pathogens followed by the activation of a coordinated response culminating in the suppression of infection. Chordates present a complex detection of pathogen associated molecular patterns (PAMPs) that leads to the activation of interferon (IFN), a key factor in activating a gene expression profile against invading pathogens. Stimulator of interferon genes (STING) is a critical protein involved in IFN- induction by some DNA and RNA pathogens upon infection, including herpes simplex virus-1 (HSV-1), *Listeria monocytogenes* and RNA viruses like vesicular stomatitis virus (VSV). STING is endoplasmic reticulum resident factor. It migrates to Golgi upon activation. In order to study STING expression and activation, we have used total RNA from HeLa cells, a human cell line derived from a cervical cancer, to amplify full length STING mRNA by RT-PCR. Full length STING PCR product was cloned in a mammalian expression plasmid and sequenced. We discovered several STING isoforms, some of them are result of alternative mRNA splicing. We have tested the ability of over-expressed STING isoforms to induce IFN- compared to a previously reported human wild type full length STING (STING Wt). Preliminary experiments have shown that these isoforms produce different levels of IFN- and NF- b. Interestingly, STING spliced forms, are unable to induce IFN- and have a different location pattern compared STING Wt. A recent report has described a novel mutant mouse strain, Goldenticket, that harbor a single nucleotide mutation (T596A) of STING that fails to produce detectable protein by western blot and acts as a null allele. It also fails to produce type I IFNs upon c-di-GMP treatment or *L. monocytogenes* infection. We are currently exploring the biological relevance of the alternative spliced STING isoforms using this novel mutant mouse strain. Overall, our data presents

interesting new aspects of STING biology that will shed light on the role of this gene in cell innate immune-biology. Future experiments should clarify the role of all STING isoforms that could help to fight against human pathogens derived diseases.

Keywords: STING, alternative splicing, IFN induction, virus-host interaction.

P-178

Citrus tristeza virus (CTV) evolving in the non-natural host *Nicotiana benthamiana*: host adaptation through serial passages?

Silvia Ambrós*[1], Josep Navarro López[1], Susana Ruiz Ruiz[2], Pedro Moreno[1]

(1) Departmento de Protección Vegetal y Biotecnología. Instituto Valenciano de Investigaciones Agrarias (IVIA). Valencia (2) Departamento de Estrés biótico. Instituto de Biología Molecular y Celular de Plantas (IBMCP).Valencia

Although natural infections of *Citrus tristeza virus* (CTV) are restricted to citrus species, agroinoculation of *Nicotiana benthamiana* (NB) plants with an infectious cDNA clone of CTV-T36 results in systemic infection of this non-natural host. Since replication in NB might affect CTV biological and molecular characteristics, we analyzed viral populations of lineages CTV-T36 and T36-GFP (with a *gfp* gene) after serial passages (P) in NB. We optimized a NB-to-NB graft-transmission procedure that enabled systemic infection of receptor plants in 1-2 months. The infection rate in serial NB-P was similar, albeit viral titer increased along P and the time for systemic infection and symptom appearance was shorter. NB-P5 and P11 virions showed the same infectivity (100%) in receptor alemow plants and induced similar symptoms in different indicator plants as did the parental citrus virions. The consensus full-genome sequence of the first CTV-NB populations was identical to the original cDNA clone. P5-CTV populations showed just 1 (T36-GFP) or 12 (T36) nucleotide (nt) changes in ORF1a regions with no functional relevance, whereas P11 populations had 16 (T36-GFP) or 17 (T36) nt substitutions along the genome, half of them involving aminoacid (aa) changes. These substitutions afected different genes (ORF1a, 1b, p33, p61, p18, p20, p23) but most were mainly concentrated in two polyprotein regions and in the p23 gene. Surprinsingly, 5 changes (3 in the ORF1a and 2 in p23) were common to both lineages at the end of the serial P. Sequencing of these regions in the rest of the P showed the gradual appearance of these mutations along the evolution of both lineages, and their further fixation in the consensus sequence of the viral populations. Most of the genetic variability observed in p23, a multifunctional protein that is a CTV pathoghenic determinant and a silencing suppressor, involved silencing mutations or aa changes that did not affect the key aa of the functional domains. Interestingly, 5 nt substitutions, 3 of which involved aa changes, were common to both lineages. They appeared

early and independently at P6-7 in both lineages and were fixed in successive P, suggesting that CTV-T36 viral populations infecting NB are different from those infecting citrus, and that some of these nt changes could reflect host adaptation. Moreover, our preliminary results have shown gradual reversion of these changes when CTV-NB P5 and P11 viral populations were returned back to citrus

Keywords: citrus tristeza virus, host adaptation, serial passages, non-natural host, consensus sequence, viral population.

P-179

Inhibition of the lymphocytic choriomeningitis virus by valproic acid

Ángela Vázquez Calvo*[1], Miguel Ángel Martín Acebes[1], Juan Carlos Sáiz[2], Nhi Ngo[3], Francisco Sobrino[1], Juan Carlos de la Torre[3]

[1] Departamento de Virología y Microbiología. Centro de Biología Molecular Severo Ochoa (UAM-CSIC).Madrid [2] Departamento de Biotecnología. INIA. Madrid [3] Department of Immunology and Microbial Science. The Scripps Research Institute. La Jolla, USA.

Valproic acid (VPA) is a short chain fatty acid commonly used for treatment of neurological disorders (Bruni and Wilder, 1979; Terbach and Williams, 2009). In a previous study, we have shown that VPA inhibited production of infectious progeny of different enveloped viruses including the arenavirus lymphocytic choriomeningitis virus (LCMV) (Vazquez-Calvo et al., 2011). In this study we have analysed the mechanisms by which VPA inhibits LCMV multiplication in cultured cells. VPA reduced production of infectious LCMV progeny and virus propagation without exerting a major blockage on either viral RNA or protein synthesis, but rather affecting the cell release and specific infectivity of LCMV progeny from infected cells. Our results would support VPA as a candidate antiviral drug to combat arenavirus infections.

References. Bruni, J., Wilder, B.J., 1979. Valproic acid. Review of a new antiepileptic drug. Arch Neurol 36, 393-398
Terbach, N., Williams, R.S., 2009. Structure-function studies for the panacea, valproic acid. Biochem Soc Trans 37, 1126-1132
Vazquez-Calvo, A., Saiz, J.C., Sobrino, F., Martin-Acebes, M.A., 2011. Inhibition of enveloped virus infection of cultured cells by valproic acid. J Virol 85, 1267-1274

Keywords: valproic acid, antiviral, LCMV, budding.

P-180

The splicing factor proline-glutamine rich (SFPQ/PSF) is essential in influenza virus polyadenilation

Sara Landeras*[1], Nuria Jorba[1], Maite Pérez[1]

(1) Department of Molecular and Cellular Biology. Centro Nacional de Biotecnología. Madrid.

The influenza A virus RNA polymerase is a heterotrimeric complex responsible for viral genome transcription and replication in the nucleus of infected cells. We recently carried out a proteomic analysis of purified polymerase expressed in human cells and identified a number of polymerase-associated cellular proteins. Here we characterise the role of one such host factors, SFPQ/PSF, during virus infection. Down-regulation of SFPQ/PSF by silencing with two independent siRNAs reduced the virus yield by 2–5 log in low-multiplicity infections, while the replication of unrelated viruses as VSV or Adenovirus was almost unaffected. As the SFPQ/PSF protein is frequently associated to NonO/p54, we tested the potential implication of the latter in influenza virus replication. However, down-regulation of NonO/p54 by silencing with two independent siRNAs did not affect virus yields. Down-regulation of SFPQ/PSF by siRNA silencing led to a reduction and delay of influenza virus gene expression. Immunofluorescence analyses showed a good correlation between SFPQ/PSF and NP levels in infected cells.

Analysis of virus RNA accumulation in silenced cells showed that production of mRNA, cRNA and vRNA is reduced by more than 5-fold but splicing is not affected. Likewise, the accumulation of viral mRNA in cicloheximide-treated cells was reduced by 3-fold. In contrast, down-regulation of SFPQ/PSF in a recombinant virus replicon system indicated that, while the accumulation of viral mRNA is reduced by 5-fold, vRNA levels are slightly increased. *in vitro* transcription of recombinant RNPs generated in SFPQ/PSF-silenced cells indicated a 4–5-fold reduction in polyadenylation but no alteration in cap snatching. Expression of mutants in the RNA binding domains of SFPQ/PSF in a recombinant virus replicon system don´t lead to a significant reduction in the CAT protein accumulation used as a reporter of total replicon activity. At present is not clear how SFPQ/PSF participates in the polyadenilation step of viral transcription but this result suggests that this activity is independent of its ability to bind RNA.

These results indicate that SFPQ/PSF is a host factor essential for influenza virus transcription that increases the efficiency of viral mRNA polyadenylation and open the possibility to develop new antivirals targeting the accumulation of primary transcripts, a very early step during infection.

Keywords: influenza virus, SFPQ/PSF, viral transcription.

P-181

The HCV RNA-polymerase NS5B is a novel target of the cellular kinase Akt/PKB

María Llanos Valero[1], Rosario Sabariegos[1], Francisco J. Cimas[1], Ricardo Sánchez Prieto[1], Antonio Mas*[1]

(1) Centro Regional de Investigaciones Biomédicas. Universidad de Castilla-La Mancha. Albacete.

HCV is a major health problem affecting more than 200 million people worldwide. Treatment strategies have to balance between the side effects of Interferon based regimens, and the resistance appearance for directed-acting HCV inhibitors. In an attempt to overcome all complications, new therapies are being directed to the cellular factors involved in RNA virus replication. The study of the interactions among proteins from viral and host origin is essential in this antiviral approach. Although there are evidences of a large number of interactions between proteins from viral and host proteins, in the case of NS5B (HCV RNA-dependent RNA polymerase*),* this number decreases dramatically, what makes this viral protein a promising candidate for further investigation. Last studies have related HCV with Akt, a member of the AGC Ser/Thr kinase. This kinase is involved in many different biological processes related to survival, trough inhibition of apoptosis and autophagy. Indeed, it has recently proposed that HCV infection in cultured cells modulates the signalling axis Akt-TSC-mTOR pathway to promote autophagy. However, the viral proteins involved in the process, the nature of the interaction, and the *in vivo* implications remind unknown.

In this study, our group has characterized the interaction between Akt and the HCV RNA-polymerase NS5B. Our data demonstrate that NS5B is a novel substrate of Akt as *in vitro* kinase with recombinant proteins assay support. In addition, physical interaction between both proteins was observed by co-immunoprecipitation assay in Huh7 Lunet cells where NS5B and Akt were overexpressed. Moreover, *in vivo* assays showed a prominent co-localization of both proteins when overexpressed in transient transfection assays in Huh7 Lunet cells, as well as in cells supporting the replication of a subgenomic HCV replicon and further transfected with a plasmid coding for exogenous Akt. Interestingly, Akt-NS5B interaction led to a change in the subcellular distribution of Akt, from a cytoplasmatic to a perinuclear signal. This is the first time to the best of our knowledge that the molecular nature of the polymerase interaction with Akt has been demonstrated. Our findings could be critical in the development of new therapies to treat HCV by targeting this cellular protein kinase.

Keywords: HCV-polymerase, host factors.

P-182

Role of p53 SUMOylation on interferon activities

Laura Marcos Villar[1], José V. Pérez Girón[2], Atenea Soto[3], Carlos F de la Cruz-Herrera[1], Valerie Lang[4], Manuel Collado[5], Anxo Vidal[3], Manuel S Rodríguez[4], César Muñoz Fontela[2], Carmen Rivas*[1]

(1) Dpto. de Biología Molecular y Celular. Centro Nacional de Biotecnología-CSIC. Madrid (2) Heinrich Pette Institute. Leibniz Institute for Experimental Virology. Hamburg, Germany (3) Dpto.de Fisioloxía y Centro de Investigación en Medicina Molecular (CIMUS). Universidad de Santiago de Compostela. (4) Ubiquitylation and Cancer Molecular Biology laboratory. Inbiomed. San Sebastián (5) Inst.de Investigación Sanitaria de Santiago de Compostela (IDIS). Complexo Hospitalario Universitario de Santiago de Compostela (CHUS), SERGAS.

Interferons (IFNs) are a critical initial defense barrier that dampens virus growth. Type I IFNs stimulate the expression of p53 and its post-translational modification by acetylation at lysine residue 320 and phosphorylation at serine 15, resulting in growth arrest and senescence. Cell infection with vesicular stomatitis virus (VSV) also upregulates p53 and induces both, its acetylation and phosphorylation, modifications that prime virus-infected cells for enhanced apoptosis.

There is growing evidence that SUMOylation regulates many host proteins involved in innate and intrinsic immunity, and that SUMO is a contributor to the regulatory process that governs the initiation of the type I IFN response. The activity of p53 can be modulated by covalent interaction with SUMO, although the result of this interaction remains unclear. Here we show that IFN treatment and infection with VSV are inducers of p53 SUMOylation. We examined the contribution of p53 SUMOylation to the cytostatic activity mediated by IFN, and evaluated the role of this modification on the antiviral activity exerted by p53. Loss of p53 SUMOylation significantly reduced its ability to induce apoptosis in response to infection with VSV, which resulted in enhanced viral replication. In addition, a p53 SUMOylation mutant also showed reduced ability to induce cell senescence in response to interferon treatment. These findings provide new insights into the mechanisms that mediate the antiproliferative and antiviral activities of type I IFNs.

Keywords: p53, SUMO, interferon, antiviral activity.

P-183

The complete genome sequence of nine ectromelia virus isolates: implications for virulence

Carla Nartuhi Mavian[1], Alberto López Bueno[1], Andreas Nitsche[2], Antonio Alcamí*[1]

(1) Dpto. de Virología y Microbiología. Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM). Madrid (2) Consultant Laboratory for Poxviruses. The Robert Koch Institute.Berlin, Germany.

Ectromelia virus (ECTV) is the causative agent of mousepox, an acute exanthematous disease of mouse colonies that occurred worlwide in the past century. Three ECTV genomic sequences are available to date: ECTV Moscow, ECTV Naval and erythomelalgia-related poxvirus. We first compared the genome sequence of the Naval strain by three different technologies: Sanger, Illumina and 454-Roche pyrosequencing. We also report eight new genomes of ECTV strains isolated from outbreaks in laboratory mouse colonies and one natural isolated from a field mouse: Cornell from North America, Hampstead, Hampstead Egg, Mill Hill, MP1, MP4, MP5 and Mou-Kre from Europe, and Ishibashi from Japan. These new ECTV genomes were pyrosequenced with a coverage ranging from 32x to 404x. Phylogenetic analysis based on the highly conserved central genomic region reveals the existence of two clades sharing at least 98% identity at the nucleotide level: the European clade, which also includes the Japanese strain Ishibashi, and an Asian-derived clade formed by the Naval and Cornell strains, and erythomelalgia-related poxvirus. The European clade contains a sub-clade formed by viruses isolated from central Europe outbreaks. A higher accumulation of mutations in immunomodulatory genes, as compared to genes located at the central region of the genome, has been detected in those viral strains isolated from animal house outbreaks. However, these changes do not compromise the integrity of the genes. On the other hand, viral strains that have been extensively passed in cell culture lost some of the immunomodulatory genes and show a substantial reduction of their virulence. Footpad infection of susceptible Balb/c mice shows a great variability among the isolates: the virulence of the central European clade strains is intermediate between that of the attenuated isolates (Mill Hill, Ishibashi and Hampstead Egg) and the prototypical virulent strains such as Moscow and Naval. Only the Hampstead and Hampstead Egg strains were found to be able to occlude viral particles within A-type inclusion bodies *in vitro* and *in vivo* due to the presence of a full length P4c gene. The role of A-type inclusion bodies in dissemination and transmission will be discussed. Our work shows that a comparison of the virulence and the gene repertoire of several poxvirus strains may provide a powerful tool to discover new immunomodulatory genes and determinants of pathogenesis.

Keywords: poxvirus, mousepox, next generation sequencing, transmission, virulence.

P-184

Sigma-1 receptor regulates early steps of viral RNA replication at the onset of hepatitis C virus infection

Martina Friederike Friesland*[1], Lidia Mingorance[1], Pablo Gastaminza[1]

[1] Departamento de Biología molecular y celular. Centro Nacional de Biotecnología. Madrid.

Hepatitis C Virus genome replication is thought to occur in a membranous cellular compartment derived from the endoplasmic reticulum (ER). The molecular mechanisms by which these membrane-associated replication complexes are for-

med during HCV infection are only starting to be unraveled and both viral and cellular factors contribute to their formation. In this study, we describe the discovery of non-opioid sigma-1 receptor (S1R) as a cellular factor that mediates early steps of viral RNA replication. S1R is a cholesterol-binding protein that resides in lipid-rich areas of the ER and in mitochondria-associated ER membranes (MAMs). Several functions have been ascribed to this ER-resident chaperone, many of which are related to Ca^{2+} signaling at the MAMs and lipid storage and trafficking. Downregulation of S1R expression by RNAi in Huh-7 cells leads to a proportional decrease in susceptibility to HCV infection, as shown by reduced HCV RNA accumulation and intra- and extracellular infectivity in single cycle infection experiments. Similar RNAi studies in persistently infected cells indicate that S1R-expression is not rate-limiting for persistent HCV RNA replication, as marked reduction in S1R in these cells does not lead to any decrease in HCV RNA or viral protein expression. However, subgenomic replicon transfection experiments indicate that S1R expression is rate-limiting for HCV RNA replication, without impairing primary translation. Overall, our data indicate that initial steps of HCV infection are regulated by S1R, a key component of MAMs, suggesting that these structures could serve as platforms for initial RNA replication during HCV infection.

Keywords: HCV, Sigma-1 Receptor, MAMs

P-185

ISG15 regulates peritoneal macrophages functionality against viral infection

Emilio Yángüez[1], Aldo Frau[2], Alicia García Culebras[2], Mariano Esteban[1], Adolfo García Sastre[3], Amelia Nieto[1], Susana Guerra*[2]

(1) Department of Molecular and Cellular Biology. Centro Nacional de Biotecnología CSIC. Madrid (2) Department of Preventive Medicine and Public Health. Universidad Autónoma de Madrid (3) Department of, Medicine, Division of Infectious Diseases. Icahn School of Medicine at Mount Sinai. New York, USA.

Upon viral infection, the production of type I interferon (IFN) and the subsequent upregulation of IFN stimulated genes (ISGs) generate an antiviral state with an important role in activation of innate and adaptative host immune responses. The ubiquitin-like protein (UBL) ISG15 is a critical IFN-induced antiviral molecule that protects against several viral infections, but the mechanism by which ISG15 exerts ist important antiviral function is not completely understood. Here, we report that ISG15 plays an important role in the regulation of macrophage responses. ISG15-/- macrophage display reduced activation, phagocytic capacity and programmed cell death in response to VACV infection. Moreover, peritoneal macrophages from mice lacking ISG15 are not able to block viral infection in co-culture experiments with VACV-infected murine embrionic fibroblast (MEFs). This phenotype is independent of cytokine production and secretion

levels, but correlates with a decrease in the phosphorylation levels of AKT of knock-out ISG15 macrophages in comparison with their wild-type counterparts. Althogether, these resuls indicate an essential role of ISG15 in the cellular immune antiviral response and points out that a better understanding of the anti-viral response triggered by ISG15 may leas to the developmetn of therapies against important human pathogen

Keywords: innate response, interferon, ISG15 and vaccinia virus.

P-186

Lipin-1 expression is required for efficient hepatitis C virion production

Lidia Mingorance*[(1)], Martina Friederike Friesland [(1)], Pablo Gastaminza[(1)]

[(1)] Departamento de Biología Molecular y Celular. Centro Nacional de Biotecnología. Madrid.

Hepatitis C Virus is a major causative agent of acute and chronic liver diseases worldwide. Increasing evidence suggests a close relationship between the HCV life cycle and lipid metabolism. In addition, it has been demonstrated that at least a portion of HCV circulates in plasma in a complex with very low-density lipoproteins (VLDL) that confers to infectious HCV particles heterogeneous and characteristically low density. In a first stage, VLDL assembly, which takes place in hepatic cells, requires active transfer of newly synthesized triglycerides to nascent apolipoprotein (apo) B-100, the major protein component of VLDL. These apoB-containing VLDL precursor particles are further loaded with lipids before they are secreted as large, lipid-loaded mature VLDL. Previous data suggest that HCV takes advantage of elements of the VLDL biosynthetic machinery, especially in terms of infectious virion production. It is controversial whether VLDL biosynthesis plays a role in infectious virion assembly. In order to address this issue, we focused our attention on Lipin-1, a phosphatidate phosphatase-1 (PAP1) enzyme that plays a key role in glycerolipid synthesis by mediating the conversion of phosphatidate to diacylglycerol, the immediate precursor of triacylglycerol. Thus, Lipin1 activity is rate-limiting for efficient VLDL secretion. We hypothesized that if VLDL production is required for efficient HCV secretion, Lipin-1 deficient cells should not be able to produce HCV virions when infected in cell culture. In order to verify this hypothesis, we silenced Lipin-1 protein expression and examined the ability of Lipin-1-deficient Huh-7 cells to support HCV infection. As expected, Lipin1-deficient cells poorly secreted apoB-containing lipoproteins, while they secreted normal amounts of apoE. Single cycle infection experiments in these cells showed a strong decrease in extracellular infectivity titers, that paralleled apoB. Our findings suggest an important role for Lipin-1 during HCV infection and are consistent with a role for triglyceride and VLDL biosynthesis in HCV assembly and secretion.

Keywords: HCV , VLDL, LIPIN-1.

P-187

A bimolecular fluorescence complementation assay to study virus-host cell protein interactions in the Rig-I like receptor pathway

M. Teresa Sánchez Aparicio*[1], Juan Ayllón[1], Adolfo García Sastre[1,2]

[1] Department of Microbiology. Icahn School of Medicine at Mount Sinai. New York, USA [2] Department of Medicine, Division of Infectious Diseases. Icahn School of Medicine at Mount Sinai. New York, USA.

The innate immune system plays a critical role in the induction of an antiviral state in viral infected cells. The secretion of several cytokines is activated with the recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs). Among PRRs, retinoic acid inducible genes (RIGI) like receptors (RLR) play an important role in the identification of RNA viruses. RIGI, upon contact with viral RNA motifs, interact with the IFN- promoter stimulator 1 (IPS-1) which triggers downstream signaling resulting in the production of cytokines such as type I and type II IFNs (1).

Some viruses have developed strategies to inhibit the induction of IFN system and survive in the host cell, by escaping from the PRRs detection system or by inhibiting the function of PRRs interacting directly or indirectly with some of the proteins that participate in the regulation of the RLR pathway (1,2). In order to study in more depth the molecular mechanisms involved in the interaction virus-host cell, we have developed a Bimolecular Fluorescence Complementation (BiFC) assay (3). The NS3/4A of Hepatitis C Virus (HCV) disrupts the subcellular localization of the complex RIGI-MAVS and MAVS-MAVS. The non structural protein 1 (NS1) from Influenza A virus (IAV) interferes in the complex formation RIGI-MAVS and TRIM25 dimerization specifically, but does not have any effect on the interaction RIGI-TRIM25. Protein V of Nipah Virus (NiV) participates in the complex RIG-I/TRIM25 and RIG-I/RIG-I. These interactions take place in different compartments in the host cells.

The use of BiFC technique has provided us a new and powerful tool to analyze known and novel protein interactions of the RLR pathway among themselves and with viral proteins in living cells. We have been able to isolate and track directly protein interactions, analyzing the localization in the host cell and the effect of these complexes in the type I IFN production.

References: 1. Versteeg G, Garcia-Sastre A (2010). Viral tricks to grid-lock the type I interferon system. Curr Opin Microbiol. 13(4):508-16
2. Garcia-Sastre, A., et al., Influenza A virus lacking the NS1 gene replicates in interferon-deficient systems. Virology, 1998a. 252(2): p. 324-30
3. Kerppola TK. Design and implementation of bimolecular fluorescence complementation (BiFC) assays for the visualization of protein interactions in living cells. Nat Protoc 2006;1:1278-1286

Keywords: influenza, BIFC, RIG-I, innate immunity.

P-188

Involvement of the RNA-binding protein Gemin5 in IRES-dependent translation

Javier Fernández Chamorro*[1], Rosario Francisco Velilla[1], David Piñeiro del Río[1], Encarnación Martínez-Salas[1]

(1) Departamento de Dinámica y función del genoma. Centro de Biología Molecular Severo Ochoa. Madrid.

Protein synthesis in various viral RNAs, including picornaviruses, is governed by a highly structured cis-acting element termed internal ribosome entry site (IRES). In addition to RNA structure, IRES function depends on the interaction with host factors, eukaryotic initiation factors (eIFs) and RNA-binding proteins termed IRES transacting factors (ITAFs). Gemin5 is a predominantly cytoplasmic protein involved in the assembly of the SMN complex and thus, in the biogenesis of the small nuclear ribonucleoproteins. This protein was identified bound to the foot-and-mouth disease virus (FMDV) and hepatitis C virus (HCV) IRES using a riboproteomic approach (1). Immunoprecipitation of UV-crosslinked complexes showed that the binding site of Gemin5 within the FMDV IRES resides in the hairpin of domain 5. Furthermore, RNA-binding assays using purified proteins revealed that this interaction depends on the C-terminal region of the protein (2). Gemin5 modulates translation activity acting as a negative regulator of IRES- and cap-dependent translation (1). In addition, Gemin5 is proteolyzed in FMDV-infected cells by the action of the L protease generating a p85 C-terminal breakdown product (3). Our work is focused to identify the proteolysis products of Gemin5 generated during FMDV infection, and to determine the role of these products in translation control. To this end, we are using Gemin5-depleted cells co-transfected with bicistronic constructs and plasmids expressing fragments of Gemin5 in conjunction with *in vitro* translation assays. In addition, we are performing a fine-mapping of the Gemin5 region responsible for IRES binding by immunoprecipitation of UV-crosslink products using purified different regions of the protein expressed in bacteria and yeast.

(1) Pacheco et al., 2009, Nucleic Acids Res 37:582
(2) Piñeiro et al., 2012, Nucleic Acids Res 41:1078
(3) Piñeiro et al., 2012 Nucleic Acids Res 40:4942

Keywords: IRES, Gemin5, FMDV, proteolysis and RNA binding proteins.

P-189

Cellular responses induced by the membrane and spike proteins of equine torovirus (BEV)

Gliselle Nieves Molina*[1], Ana María Maestre Meréns[2], Susana Plazuelo Calvo[1], Dolores Rodríguez Aguirre[1]

(1) Dpto. de Biología Molecular y Celular. Centro Nacional de Biotecnología CSIC. Madrid (2) Department of Microbiology. Mount Sinai School of Medicine. New York, USA.

Toroviruses are enteric positive-stranded RNA viruses classified in the *Coronaviridae* family, *Torovirinae* subfamily, of the *Nidovirales* order. Four torovirus species have been described: bovine torovirus (BToV), porcine torovirus (PToV), human torovirus (HToV) and equine torovirus (EToV). The EToV, Berne virus or BEV, prototype member of the *Torovirus* genus induces apoptosis in infected cells at late time postinfection (1). This process involves protein synthesis inhibition through the activation of the double-stranded RNA-dependent protein kinase (PKR), and the caspase cascade, through both, the cell death receptor (extrinsic) and the mitochondrial (intrinsic) pathways. In this work we have studied the potential involvement of the nucleocapsid (N), membrane (M) and spike (S) BEV proteins in this process. Also, as the M and S proteins are produced in the endoplasmic reticulum (ER), we wanted to determine the possible activation of the PKR-like ER kinase (PERK) and other effectors of ER stress response. We generated recombinant vaccinia viruses (rVV) that express the N, M and S proteins and used them to infect HeLa, 293T and BHK-21 cells. In addition, we used rVV that express dominant negative forms of the eIF2 and PKR to coinfect HeLa cells. We observed that the M and S proteins induced the apoptotic process, through phosphorylation of the eIF2 and protein synthesis inhibition, as well as the activation of the executor caspases, in all cell lines analyzed. We also determined that both the extrinsic and intrinsic pathways were activated upon expression of the M or S proteins. In addition, we observed that the coexpression of the dominant negative forms of eIF2 and PKR reduced dramatically the apoptosis induced by the M protein, but only a partial reduction was observed in the case of the S protein. Finally, we observed that BEV, as well as the M and S proteins expressed individually, activated the ER stress response through the splicing of the X box-binding protein 1 (XBP-1) pre-mRNA by the inositol requiring enzyme-1 (IRE1). Together, these results confirm that the M and S proteins are implicated in the apoptotic process induced by the BEV virus, and show that these proteins are also responsible for triggering the cellular ER stress response in BEV infected cells.

1. Maestre, AM, Garzón. A., Rodriguez, D. 2011. "Equine torovirus (BEV) induces caspase-mediated apoptosis in infected cells". PloS One 6 (6), e20972. 2011

Keywords: Apoptosis, torovirus, endoplasmic reticulum stress.

P-190

Characterization of the chemokine binding proteins E163 and 35-kDa from ectromelia virus

Antonio Alcamí[1,2], Haleh Heidarieh*[1]

(1) Dpto. de Virología y Microbiología. Centro de Biología Molecular Severo Ochoa (CSIC-UAM). Madrid (2) Faculty of Medicine. University of Cambridge . UK.

Poxvirus are large DNA viruses that encode approximately 100 genes dedicated to evade the im-

mune system of their hosts. Ectromelia virus (ECTV) is the causative agent of mousepox and is used as a model for variola virus to study viral pathogenesis and the interaction of viruses with the immune system. One of the strategies that have been adopted by poxviruses to sabotage the immune defenses is the expression of viral secreted chemokine binding proteins (vCKBPs) that have no sequence similarity to cellular counterparts. Chemokines interact with both their specific receptors and cell surface glycosaminoglycans (GAGs) via distinct binding sites. Two vCKBPs have been shown to inhibit chemokine activity by different mechanisms of action. The 35-kDa vCKBP from vaccinia virus (VACV) binds the receptor binding site of a broad range of chemokines. The E163 protein from ECTV strain Naval is a vCKBP orthologue of the VACV A41 protein and they have structural similarities to the 35-kDa protein. E163 binds with high affinity a limited number of CC and CXC chemokines trought their GAG binding domain, suggesting that E163 may prevent the formation of the chemotactic gradient required for leukocyte migration to the sites of infection. In addition, the A41 protein interacts with GAGs to be retained at the cell surface. The purpose of the present work was to determine the mechanism by wich E163 inhibits chemokine activity, as compared to the 35-kDa vCKBP, and its contribution to pathogenesis and immune modulation in a mouse model of poxvirus pathogenesis. In order to characterize the GAG binding site of E163 we have expressed seven mutant proteins in the baculovirus system. We found that the ^{141}KTKDFMK^{147}, ^{152}LKPRDFKT^{159} and ^{213}RKILKKKF^{220} motifs correspond to the GAG binding sites of the protein. Chemokine and GAG binding affinity constants were determined by surface plasmon resonance using a Biacore X100 biosensor. The mutant proteins have lost totally or partially their ability to interact with GAGs but they are still binding chemokines. We have generated a recombinant virus deleted in the *gene E163* of ECTV (ECTVDE163) and confirmed by Illumina massive sequencing the absence of other mutations in the genome. ECTVDE163 and an ECTV mutant in the 35-kDa *gene* will be tested *in vivo* in infected mice to determine their role in viral pathogenesis.

Keywords: ectromelia virus, chemokine binding proteins, glycosaminoglycans, viral pathogenesis, chemokines and immune system

P-191

Microarray technology applied to the study of natural viral communities

Fernando Santos*[1], Manuel Martínez García[1], Mercedes Moreno Paz[2], Ramón Roselló-Móra[3], Víctor Parro[2], Josefa Antón[1]

[1] Departamento de Fisiología, Genética y Microbiología. Universidad de Alicante. [2] Departamento de Ecología Molecular. Centro de Astrobiología [CSIC-INTA]. Torrejón de Ardoz, Madrid. [3] Marine Microbiology Group. Institut Mediterrani d'Estudis Avançats [CSIC-UIB] Mallorca.

Microarray technology was introduced 16 years ago in the field of Microbial Ecology to study the microbial composition in natural samples by using

probes for 16S rRNA genes from nitrifying bacteria. Since then, microarrays have been applied not only to the study of the phylogenetic groups present in a sample ("phylochips") but also to the study of the genetic expression (functional microarrays). In the field of Virology, microarrays have been used: (i) as a tool to analyze the genetic diversity in related viruses and (ii) to study the viral genetic expression in infected cultures or natural samples.

The Molecular Microbial Ecology group from the University of Alicante (in collaboration with the Molecular Ecology Group at Centro de Astrobiología INTA-CSIC) was pioneer in the use of microarrays for the analysis of the genetic expression in natural samples. Microarrays containing viral DNA from a hypersaline water sample (the CR30 crystallizer from solar salterns "Bras del Port", located in Santa Pola, Alicante, Spain) were hybridized against total cDNA obtained from the same microbial community. This approach allowed a "metaviriotranscriptomic" analysis where only viral transcripts were analyzed, reducing the efforts that suppose the study of the total cDNA and the assignment of a transcript to viral sequences that, in most cases, are related to hypothetical proteins. This study revealed that the halophilic viral community ("haloviruses") was highly active at the time of sampling and that viral groups with the higher expression could be related to the high G+C content prokaryotes present in the sample (extremely halophilic *Bacteroidetes* and some extremely halophilic *Archaea* from the family *Halobacteriaceae*).

Currently, we are carrying out studies where microarrays are used to detect virus-hosts systems in the environment. For this purpose, chips containing immobilized halovirus genomes are being hybridized against DNA from individual cells, separated by Single Cell Genomics technology, directly from the natural sample. Hybridization between a viral genome and cellular DNA could be indicating that, at the time of sampling, the cell was infected. These results could be very useful for further studies of virus-host dynamics in nature and address the analysis of natural samples without the cultivation of the virus-host system which, in many cases, represents a minor fraction of the microbial community.

Keywords: microarray, hypersaline, halovirus

P-192

Study of pathogenicity factors Involved in the outcome of the infection by influenza virus: viral markers and possible role of genetic markers of the patient

Ana Falcón*[1], Ariel Rodríguez[1], María Teresa Cuevas[2], Inmaculada Casas[2], Francisco Pozo[2], Juan Ortín[1], Amelia Nieto[1]

(1) Department of Cellular and Molecular Biology. CNB-CSIC.Madrid (2) Department of Virology. CNM-ISCIII. Majadahonda, Madrid.

Influenza virus infections are a major cause of respiratory disease in humans, and they cause annual epidemics and occasional pandemics with potentially fatal outcome. The differences in the severity of the disease among individuals infected by influenza viruses could be due to pre-existing health conditions, to genetic factors, to differences in the virulence of the circulating viruses or a combination of these elements. As a model for influenza virus virulence, we have studied the infection with A(H1N1)pdm09 viruses. The biological properties of viruses isolated from a patient showing mild disease (M) or from a fatal case (F), both without known comorbid conditions were compared *in vitro* and *in vivo*. The F virus presented faster growth kinetics and stronger induction of cytokines than M virus in human alveolar lung epithelial cells. In the murine model *in vivo*, the F virus showed a stronger morbidity and mortality than M virus. Remarkably, a higher proportion of mice presenting infectious virus in the hearts, was found in F virus-infected animals. Altogether, the data indicate that strains of pH1N1 virus with enhanced pathogenicity circulated during the 2009 pandemic. Genetic characterization of M and F viruses was performed by ultrasequencing of purified virion RNAs. The two viruses differed in 29 nucleotides distributed over all the RNA segments, which produce 9 amino acids changes. Residues HA 127L, PB2 221T and PA 529N were only detected in F virus and appeared as particularly interesting. To evaluate the potential role of aminoacids HA 127L, PB2 221L and PA 529N as virulence markers, we have generated recombinant influenza Cal/04 (pandemic reference strain) viruses carrying HA S127L, PB2 A221T, PA D529N mutations (single, double or triple mutants). Virulence of these viruses is being analyzed *in vitro* and *in vivo*. With regard to knowledge of the host characteristics, potential influenza virus host determinants, which affect the patient´s response to the infections, have been studied recently. One of these potential host genetic determinants is a deleted form of chemokine receptor 5 (*CCR5*), *CCR5 32*, which correlates with a severe progression of the infection. Thereby, in addition to the above described analysis of differences in virulence of viruses isolated from patients showing different pathology, examination of *CCR5* genotype of the patients was performed. This analysis revealed that the F virus-infected patient was homozygous for *CCR5* 32**.**

Keywords: influenza virus, pathogenesis, virulence markers, host genetic markers.

P-193

Interferon a regulates the transcriptional activity of the LTR region of the caprine arthritis encephalitis virus

Rafael N. Añez*[1], Ana Doménech[1], Diego Castillo[2], Ricardo A. Roa Castellanos[1], Brian Murphy[2], Esperanza Gómez Lucía[1]

(1) Dpto. Sanidad Animal. Facultad de Veterinaria UCM. Madrid (2) Pathology, Microbiology and Immunology. School of Veterinary Medicine, UC-Davis, USA.

Caprine arthritis encephalitis virus (CAEV) is a lentivirus that infects goats and sheep and causes mainly arthritis and encephalitis, but also chronic mastitis and interstitial pneumonia.

As seen in other studies, the proviral LTR of retrovirus can be influenced by many different cellular factors. Our aim was to analyze how type I interferons may modify transcriptional activity of the CAEV promoter U3 region located within the proviral LTR.

Using Clustal Omega software, our research group has identified the *interferon sequence response elements* (ISRE) 5'- A/G NGAAANNGAAACT in the U3 region within the proviral LTR of four different CAEV field isolates. These ISRE sites have been shown in other cellular promoters to respond to interferon molecules. In order to evaluate whether or not this lentiviral ISRE site is transcriptionally functional, the U3 region of these four CAEV isolates was cloned into the the pBlueTOPO plasmid 5' to the -galactosidase (-gal) reporter gene. To study whether these ISRE were functional, the human 293T cell line was transfected using JetPrime with these recombinant reporter plasmids and incubated with tenfold dilutions of IFN (A/D) (a hybrid that crosses the species barrier; 0.04-40 IU/ml). Experiments were performed in triplicate. The expression of the -gal gene driven by the CAEV LTR was assessed colorimetrically by a standard biochemical assay (Sambrook, 1989). Exposure of 293T cells transfected with the CAEV- -gal constructs to IFN for 48 h resulted in twofold promoter activation in the range 0.4 IU/ml-4 IU/ml relative to transfected but untreated cells. The expression of -gal severely dropped when cells were treated with 40 IU IFN /mL. Differences were observed between the plasmids. These results demonstrate that the ISRE sites in the U3 region of the proviral LTR of CAEV field isolates are functional, although the complex role of the ISRE site in lentiviral pathogenesis remains incompletely understood.

Keywords: Interferon, CAEV, ISRE, lentivirus, LTR.

P-194

Hepatitis C virus hijacks a selected set of cellular decapping activators to establish infection

Nicoletta Scheller[1], Gemma Pérez Vilaró*[1], Laura Olivares Boldú[1], Verónica Saludes[1], Juana Díez[1]

[1] Department of Experimental and Health Sciences, Molecular Virology. Universitat Pompeu Fabra. Barcelona.

A growing body of evidence indicates that a complex interplay exist between the eukaryotic mRNA degradation machinery and RNA virus expansion. Besides the anticipated mechanisms to avoid elimination, some RNA viruses subvert components of these pathways to promote their propagation. We have previously shown that the hepatitis C virus hijacks the cellular decapping activators DDX6, LSm1 and PatL1 to promote its translation and replication. Importantly this role was specific and not related to the decapping and degradation processes *per se* since the decapping enzyme Dcp1/Dcp2 and the 5´-3´ Xrn1 exonuclease

that degrades decapped mRNAs did not have it. Whether additional decapping activators might exert a similar role on the HCV lifecycle remained unknown. Here we analyzed the effect of the other two decapping activators, Edc3 and Edc4. Infection of Edc3-depleted cells resulted in a 10-fold reduction of intracellular HCV RNA accumulation and virus production, while Edc4 depletion had no effect. Moreover, infection of cells overexpressing Edc3 enhanced HCV propagation. In contrast, when HCV RNA replication was fully established, we did not observe a significant reduction in HCV production when Edc3 or DDX6 were depleted, indicating that these decapping activators are critical for establishment of HCV infection. Next we tested which steps of the HCV lifecycle depend on Edc3. Edc3-depletion inhibited replication of a dicistronic subgenomic HCV replicon that supports only HCV translation and replication, indicating that Edc3 promotes these early steps of the HCV lifecycle. To specifically test a putative role on translation we used a full-length non-replicating HCV-luciferase reporter RNA. Luciferase expression was inhibited by 2-fold under Edc3 knockdown conditions. Importantly, Edc3 co-immunoprecipitated with HCV RNA and HCV NS3 protein, supporting a direct and specific interaction with HCV components. Finally, like previously observed for DDX6, LSm1 and PatL1, HCV infection decreases the localization of Edc3 and Edc4 in P-bodies, suggesting a dynamic interplay between HCV and these structures. Taken together, our results show that to establish infection HCV specifically requires a subset of decapping activators to promote the early translation and replication steps.

Keywords: HCV, decapping activators, Edc3.

P-195

Specific association of different yeast L-A viruses and their killer toxin-encoding M dsRNA satellites suggests co-evolution

Nieves Rodríguez Cousiño*[1,2], Pilar Gómez[2], Rosa Esteban[2]

(1) Departametno de Microbiología y Genética. Universidad de Salamanca. (2) Instituto de Biología Funcional y Genómica (IBFG). CSIC. Univ. Salamanca.

Saccharomyces cerevisiae L-A virus (ScV-L-A) is a cytoplasmic-persisting dsRNA virus of the *Totiviridae* family. The unsegmented genome (4.6 kb) encodes two virion proteins: the major structural protein (Gag) and a minor Gag-Pol fusion protein, responsible for the transcriptase and replicase activities associated with L-A virions. Many yeast strains harbouring L-A also carry dsRNA satellites called M, which code for different killer toxins, K1, K2, K28 or Klus. The L-A present in Klus strains showed poor hybridization to L-A probes from laboratory K1 strains, suggesting substantial differences. We have characterized this new L-A variant, which we named L-A-lus (1). We studied the distribution of this L-A-lus variant in around 30 wine strains from different oenological regions. All selected strains (killer or non killer) carried L-A. Apart from L-A-lus we found another L-A variant, L-A-2, associated with M2 in K2 strains. No strain carrying L-A was found. We cloned and sequenced L-A-2. L-A, L-A-lus and L-A-2 show around 75% identity at the nucleotide

level, that raises to 86% in the amino acid composition of Gag or Gag-Pol. Two regions in their genomes, however, are 100% identical; the frameshifting region between Gag and Pol and the encapsidation signal, implying the importance of these two *cis* signals in the virus life cycle. To know more about these L-A variants we constructed laboratory strains that carry L-A-lus or L-A-2 alone, or with their respective M satellites. We found that L-A-lus and L-A-2 show higher resistance than L-A to growth at high temperature or to *in vivo* expression of the 5'-3' *XRN1/SKI1* exonuclease. Also in *S. cerevisiae* strains with a RNA interference reconstituted pathway L-A-lus or L-A-2, though diminished, were much less affected than L-A by the expression of Dcr1p and Ago1p endonucleases. This higher resistance of L-A-lus and L-A-2 to the different traits analyzed may explain their prevalence in nature over L-A. We also studied the helper activities of L-A-lus and L-A-2 over different dsRNA satellites. Our data show that distinct M killer viruses are specifically associated to L-As with different nucleotide composition, suggesting co-evolution.

(1) Rodríguez-Cousiño N, Gómez P, Esteban R. L-A-lus, a new variant of L-A totivirus in wine yeasts associated to Klus killer toxin-producing Mlus dsRNA. Possible role of satellite RNAs encoding killer toxins on the evolution of their helper viruses. Appl. Env. Microbiol. (submitted).

Keywords: yeast dsRNA viruses, killer toxin, M dsRNA satellites.

P-196

Human norovirus in Gipuzkoa. From Minerva variant in 2009 to Sydney variant in 2012

Ainara Arana*[1], Milagrosa Montes[1], Luis D. Piñeiro[1], María Gomariz[1], María Soledad Zapico[1], Gustavo Cilla[1], Emilio Pérez Trallero[1]

(1) Department of Microbiology. Hospital Universitario Donostia-Biodonostia.

Introduction. Human noroviruses (HNV) are the major cause of acute gastroenteritis (AGE) outbreaks and of sporadic AGE worldwide, and after rotavirus, the leading cause of severe AGE in infants. HNV are RNA viruses genetically diverse (5 genogroups, >40 genotypes), which evolve using molecular mechanisms that remind those of influenza viruses. Every 2-3 years a new variant of genogroup II genotype 4 (GII.4) emerges and is distributed worldwide causing an increase in the disease incidence (pandemic). The aim of this work was to investigate the role and genotype distribution of HNV causing AGE in Gipuzkoa.

Methods. Between 2009 and 2012, an unselected sample of 715 feces positives to HNV obtained from 450 children <2 years old, 141 children 2-14 years old and 124 adults patients mainly from institutional outbreaks, were analysed. HNV detection was performed by RT-PCR(1), and subsequently cDNAs were stored at –40ºC. Thereafter, a selection for genotyping up to 10 positive samples per month was made. Molecular characterization of these strains was performed by par-

tial sequencing of capsid and polymerase (PrPd) genes (2,3). A phylogenetic analysis was conducted using the Clustal X and Mega5 programs.

Results. The 715 HNV episodes were distributed in all but one month of the study (47/48), although cold months showed high number of detections. Genotyping was successful in 238/372 HNV episodes (capsid in 204, PrPd in 198, both genes in 164). Four strains were GI (1,7%) and 234 GII (98,3%). Based on the capsid gene of GII, 7 genotypes were detected: GII.4 (73%), GII.3 (23%), GII.6 (1%), and others (3%). GII.4 strains predominated during the four years of study, both in children and adults. GII.3 strains were mainly detected in infants aged 1 year, and were more frequent in 2009 and 2012. Most of the GII.4 strains of 2009 clustered with the Minerva variant (USA 2006), those of 2011 with the New Orleans variant (USA 2010), dividing the strains of 2010 between the two already mentioned. Since late August 2012, the new Sydney variant (Australia 2012) was the main GII.4 strain detected.

Conclusion. GII.4 strains predominated in Gipuzkoa but changes in this genotype were frequent. The variants causing global norovirus pandemics were found in Gipuzkoa. HNV was a frequent cause of AGE in patients of all ages.

(1) Maguire AJ, JCM 2009;37:81; (2) Buesa J, JCM 2002;40:28549 (3) Puustinen L, Epidemiol Infect. 2012;140:268

Keywords: norovirus, epidemiology, variant.

P-197

Emergence of G12[P8] rotavirus, an unusual genotype, in the Basque Country during the seasonal epidemics of 2010-2012

Ainara Arana*[1], Milagrosa Montes[1], María Gomariz[1], Felicitas Calvo Muro[1], Ildefonso Perales[1], Gustavo Cilla[1]

[1] Microbiology. Hospital Universitario-Biodonostia. San Sebastián.

Introduction. Human group A rotaviruses (RV) are the leading cause of severe acute gastroenteritis (AGE) in children worldwide. Currently, 27 G-genotypes and 35 P-types are known, of which G-types G1-G4 and G9 are globally considered the major genotypes, causing >90% of rotavirus infections in Europe. G12 is an unusual RV, but it contains genetic and epidemiological features that suggest it has the potential to join the group of major rotaviruses. Unexpectedly, G12 strains were predominant during the 2010-11 epidemic in Gipuzkoa (1), and continued showing dominance in 2011-12. The aim of this work was to describe these two epidemics caused by the unusual G12 RV.

Methods. The presence of group A RV was studied (July 2010-June 2012) in faeces of children with AGE aged <5 years old for whom stool culture was requested. RV detection was performed by ELISA (Dako u Oxoid). Positive samples were suspended in B199 medium and stored at -80°C until analysed for G and P types through multiplex nested RT-PCR methods (1).

Results. Samples of 5255 children from Gipuzkoa were analysed, and RV was detected in 525 (10%), corresponding to 475 AGE episodes. G and/or P-type was achieved in 440 strains, representing 98% of samples in which enough amount was able for genotyping (450). In 418 strains both G and P type were obtained. G12 was dominant in both seasons (62% and 80% respectively) followed by G1 (31,5%) in 2010-11 and G9 (14%) in 2011-12. Circulation of G12 lasted for 7 months in both epidemics (Sept10-March11) and (Oct11-April12), having 6 months in between with no detections. 24 (16,5%) and 30 (12,5%) children were hospitalized because of AGE due to G12 RV in 2010-11 and 2011-12, respectively. G12 RV circulated widely in Gipuzkoa, incidence being similar in rural and urban areas (100 and 110 cases/10000 children <5 years old of municipalities of <50000 and >50000 inhabitants, respectively). In the 2011-12 epidemic, faecal samples from Álava and Vizcaya were analysed, G12 being detected in 10/14 and 8/10 samples, respectively.

Conclusions. G12 Rotavirus caused the annual seasonal epidemics occurred in Gipuzkoa in 2010-11 and 2011-12, and probably in 2011-12 in the whole Basque Country. To our knowledge, these are the first G12[P8] epidemics reported in Europe and they confirm that G12 RV could cause high impact seasonal epidemics in developed regions. G12 may soon become a major human rotavirus genotype.

(1) Cilla G et al. Epidemiol Infect 2013

Keywords: rotavirus, G12, epidemiology, emerging viruses.

P-198

Generation of human recombinant prions. Model for understanding the Gerstmann–Sträussler–Scheinker syndrome

Saioa R. Elezgarai*[1], Natalia Fernández Borges[1], Hasier Eraña[1],Ester Vázquez[2], Chafik Harrathi[1], Sonia Veiga[2], Larisa Cervenakova[3], Paula Saá[3], Witold Surewicz[4], Olivier Andreoletti[5], Jesús R. Requena[2], Joaquín Castilla[1,6]

(1) Department of Proteomics. CIC bioGUNE. Derio, Vizcaya (2) CIMUS Biomedical Research Institute & Department of Medicine. University of Santiago de Compostela-IDIS. (3) Jerome H. Holland Laboratory for the Biomedical Sciences. American Red Cross. Rockville, USA (4) Department of Physiology & Biophysics. CEAFA. Cleveland, USA (5) UMR INRA ENVT 1225, Interactions Hôte Agent Pathogène. Ecole Nationale du Veterinaire. Toulouse, France (6) IKERBASQUE. Basque Foundation for Science. Bilbao.

Human transmissible spongiform encephalopathies (TSEs) or human prion diseases belong to a group of fatal neurodegenerative disorders that includes kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), and fatal familial insomnia (FFI) among others. Human TSEs may occur sporadically or on a genetic or iatrogenic basis. Gerstmann-Sträussler-Scheinker disease (GSS) is a genetically determined TSE characterized by adult onset of memory loss, dementia, ataxia, and pathologic deposition of amyloid-plaques in the brain. It is caused by a range of mutations within the open reading frame of the prion protein (PrP)

gene (*PRNP*). The P102L substitution was the first discovered and the most common mutation. The consequences of pathological mutations to the human PrP conformation and their effects on pathogenesis are still poorly understood.

The aim of this study is to generate a useful model for understanding GSS. To achieve this, Protein Misfolding Amplification (PMCA) is being used, as it mimics PrP^{C} to PrP^{Sc} conversion *in vitro* with accelerated kinetics. Specifically, PMCA based on recombinant PrP (recPMCA) is being used instead of brain-derived PrP. A substrate based on P102L mutated human recombinant PrP was subjected to serial rounds of recPMCA until a recombinant human GSS P102L PrP^{Sc} was generated spontaneously. The recombinant prion was unable to propagate the misfolding to a wild type human recombinant PrP based-substrate by recPMCA. These results are comparable to *in vivo* results using both non-human primates and rodents as recipients for different GSS isolates from human patients. We are currently performing different biochemical, biological and structural studies trying to characterize the new recombinant PrP^{Sc} as a model for understanding Gertsmann-Straussler-Scheinker syndrome.

Keywords: prion, GSS, PMCA.

P-199

Bexarotene as a possible drug for the treatment of prion disease

Alejandro M. Sevillano*[1], Bruce Onisko[2], Natalia Fernández Borges[3], Manuel Sánchez-Martín[4], Joaquín Castilla[3], Jesús Rodríguez Requena[1,5]

(1) Department of Molecular Neuropathology. CIMUS, Biomedical Research Institute, University of Santiago de Compostela-IDIS (2) OniPro. OniPro. Kensington, USA (3) Proteomics. 3CIC bioGUNE, Parque Tecnológico de Bizkaia. (4) Unidad de Generación de OMGs. S.E.A. University of Salamanca. (5) Medicina. University of Santiago de Compostela.

Prions are a novel class of pathogens devoid nucleic acids. While the cellular prion protein, PrP^{C}, is a ubiquitous membrane protein, its abnormally folded conformation, PrP^{Sc}, can aggregate to form a b-amyloid. This aberrant conformation causes the transmissible spongiform encephalopathies (TSEs), fatal neurodegenerative ailments affecting humans and animals. There are different human TSEs, characterized by rapidly progressive dementia, personality changes, ataxia, seizures and finally, death. Prion disease may have a genetic, infectious or sporadic origin. Inherited prion diseases are associated with mutations in the *PRNP* gene. Infectious prion disease can result from dietary or iatrogenic transmission of the very resilient prions, and sporadic cases are presumably initiated by a rare misfolding event. There is currently no treatment for prion diseases. Once acquired, all forms of prion disease are potentially transmissible.

CJD and Alzheimer´s disease (AD) share pathological molecular pathways, namely, accumulation of misfolded protein in the brain. Recently, Cramer *et al*. (*Science, 335:1503-6*) have demonstrated that the bexarotene has an important effect in inhibiting and reverting beta-amyloid plaque formation in murine models of AD, with an important cognitive recovery. Bexarotene, a licensed drug prescribed to treat some forms of skin cancer, acts by increasing ApoE expression in the brain. ApoE is a component of lipoproteins which promotes proteolytic degradation of soluble forms of A .

In turn, we have evidence showing that ApoE binds strongly to PrP^{Sc}. Taken all this together, we propose that bexarotene might be effective in blocking propagation of prions in the brain.

We have started a pilot experiment supplying 100mg/kg/day bexarotene, by oral administration, to transgenic mice expressing PrP of the bank vole with the 109I polymorphism (tgBVPrP109I), which develop a spontaneous prion disease. We initiated treatment when mice were ~50 days old, when infectivity is first detected in their brains. At the present moment the mice have undergone 100 days of treatment and no toxicity has been observed, which confirms the feasibility of this approach and suggests that we will be able to continue the treatment beyond the incubation time of prion disease in these mice. This study should provide a proof of principle of the capacity of bexarotene to inhibit PrP^{Sc} accumulation and, consequently, prevent the clinical signs of prion disease.

Keywords: prion, CJD, AD, beta-amyloid, ApoE, bexarotene.

P-200

Performance different of diagnostic tests in a mumps outbreak

Lisbeth Gonçalves De Freitas*[(1)], Silvia Rojo[(1)], Elena Alvarez[(1)], Gabriel March[(1)], Mar Justel[(1)], Cristina López[(1)], Ana Rodríguez[(1)], Eleda Coletta[(1)], Ana Avila[(1)], Raúl Ortiz De Lejarazu[(1)]

(1) Departamento de Microbiología e Inmunología. Hospital Clínico Universitario de Valladolid.

Introduction. Mumps outbreaks of different magnitude occur in Spain and Europe some years. Diagnosis is essentially clinical and microbiological tests addressed to confirm the clinical picture and rule out other diagnoses. This paper presents the results of techniques found in the cases referred to microbiology and proposes the best diagnostic options.

Material and methods. We analyzed 72 patients with mumps by EIA for IgG and IgM of mumps virus (Vircell®), IgG and IgM against CMV (Vidas®), IgG and IgM to VCA EB (Trinity Biotech®); heterophile antibodies for EB and FC antibodies against adenovirus. Samples of serum, urine and saliva were also sent to CNMVIS (Majadahonda) for specific PCR. Age range was 11 months - 55 years, mode was 21 years, majority of cases were 11 - 30 years (79.2%).

Results. 59 out of 72 patients with clinical mumps were confirmed by microbiological techniques. All patients made exception of 4 showed specific mumps IgG. Sera showed IgM demonstrative of acute infection (18-20%). There was good agree-

ment between IgM-IgG results of both centers. Adenovirus and EBV serology did not add other diagnosis different from mumps. IgG positive against EBV VCA was present in 91.3% patients. One patient (29 years) showed IgM against EBV and another (23 years) showed IgM against CMV, but both had presence of mumps virus in saliva by PCR. CMV IgG was present in 47,8% patient, with only one case of CMV IgM positive and negative for all markers for mumps.

PCR in saliva was the technique that allowed a greater number of diagnoses; 55 out of 68 samples (80.9%). Among the 13 negative patients for PCR in saliva, specific mumps IgM was found in two of them. In other four patients that referred only urine and serum, the presence of IgM allowed diagnosis in one of them and the urine PCR allowed the diagnosis in another case. Only 5 samples out of 42 urines were positive by PCR (10,9%). All those samples but one, were positive in saliva samples.Except one CMV case no other confounding infection has been confirmed among mumps cases referred to hospital.

Conclusions. PCR in saliva was the best method for microbiological confirmation of majority of mumps cases while specific IgM confirmed 25% cases with or without IgG. Therefore

cost effectiveness diagnosis approach in mumps outbreaks among vaccinated population could begin by assessing first presence of IgM in serum and IgM negative individuals analized by PCR of saliva.

Keywords: mumps, Outbreak, PCR.

P-201

Cryo-electron microscopy of HHIV-2: a new salt-loving archaeal virus

Daniel Badia Martinez*[1], David Gil-Carton[1], Salla K. Jakkola[2], Hanna M. Oksanen[2], Dennis H. Bamford[2], Nicola G.A. Abrescia[1,3]

(1) Department of Structural Biology Unit. CICbioGUNE. Derio, Vizcaya (2) Institute of Biotechnology and Department of Biosciences. University of Helsinki. Finland (3) IKERBASQUE. Basque Foundation for Science. Bilbao.

Haloarcula hispanica icosahedral virus 2 (HHIV-2) is a recently discovered lipid-containing virus that infects the halophilic archaea *Haloarcula hispanica.* So far very little is known about Archaea infecting viruses and only few archaeal viruses have been structurally characterised: STIV, ASFV1 and SH1. Based on the recent idea that viruses can be classified using coat protein fold and virion architecture it would be very useful to compare the different solutions that evolution has provided for the assembly of viruses infecting archaeal, bacterial and eukaryotic cells.

A preliminary three-dimensional reconstruction of HHIV-2 at ~12Å resolution (for the capsid as judged by the FSC=0.5) was obtained by projection matching (XMIPP program) using as initial reference a re-sized map of SH1 virus filtered to 80Å. The 3D reconstruction shows an icosahedral virus with a size of ~710Å facet-to-facet, 770Å vertex-to-vertex and 725Å edge-to-edge. Whereas the 5 capsomers composing the icosahedral

asymmetric unit are structurally similar to those of SH1 and arranging with the same lattice (*p*T28), the pentameric structures protruding ~100Å from each of the twelve vertices of HHIV-2 are strikingly different from SH1 dimeric spikes. The density of the vesicle at the five-fold apices is stronger and suggesting a plug-like structure connecting with the above vertex complex.

Currently our efforts are directed at improving the resolution of the reconstruction and at deepening the analysis of the virus density.

Acknowledgements. We thank Bibiana Peralta for initial assistance with data processing. We also acknowledge Magda Wojtas, Marina Ondiviela, Sari Korhonen and Soile Storman for their skillful assistance.

Keywords: archaea, virus structure, cryo-Electron microscopy, evolution.

P-202

Emerging mosquito-borne flaviviruses in Europe: a growing concern

Miguel Ángel Jiménez Clavero*[(1)], Francisco Llorente[(1)], Elisa Pérez Ramírez[(1)], Jovita Fernández Pinero[(1)], Maia Elizalde[(1)], Ramón C. Soriguer[(2)], Jordi Figuerola[(2)]

(1) Centro de Investigación en Sanidad Animal (CISA). INIA. Valdeolmos, Madrid (2) EBD. CSIC. Sevilla.

Vector-borne diseases are gaining importance in the context of climate and global changes. Last decade has witnessed an upsurge in the incidence and geographic spread of different mosquito-borne flavivirus infections having wild birds as reservoirs. Relevant examples are the worldwide spread of West Nile virus (WNV), Usutu virus (USUV) in Europe, Zika virus in Indian Ocean countries, Tembusu and Baiyangdian viruses in China. Their impact not only in animal health, but also as actual or potential zoonotic agents, is of concern.

In Europe, mosquito-borne flaviviruses were considered rare and exotic not so long ago. However, more recently they are getting more common, especially in certain areas where eco-climatic conditions favor their circulation. The most relevant example is WNV: after a long absence from Europe, this virus reemerged in this Continent in the late 1990's and since then spread relentlessly leading to a situation in which up to 17 European countries have reported clinical cases recently, with a total of 907 cases in 2012 (1). In parallel, an increasingly complex genetic heterogeneity of WNV has been noticed, with several lineages and sublineages circulating in different parts of Europe. As a further step in complexity, the spread of USUV since its first record in 2001 in Central Europe is also of concern (2). USUV is similar to WNV not only at the genetic and antigenic levels, but also in life cycle, transmission and host range. More recently (2010), a third mosquito-borne epornitic flavivirus, Bagaza virus (BAGV) was identified as the cause of disease outbreaks in birds (partridges and pheasants) in Southern Spain. Circulation and spread of WNV, USUV and BAGV in Europe is a new scenario that merits further attention, in particular regarding their efficient diagnosis, surveillance and control. This presentation

will summarize the most recent advances made in our laboratory on pathogenesis, diagnosis, ecology and epidemiology of these three flaviviruses.

(1) ECDC, West Nile virus situation update 30/11/2012 http://ecdc.europa.eu/en/healthtopics/west_nile_fever/West-Nile-fever-maps/Pages/index.aspx
(2) Vazquez A, et al (2011). Usutu virus: potential risk of human disease in Europe. Euro Surveill. Aug 4;16(31)
(3) Agüero M, et al. (2011). Bagaza virus in partridges and pheasants, Spain, 2010. Emerg Infect Dis. 17(8):1498-501
Acknowledgments: Grants EU HEALTH 2010.2.3.3-3 Project 261391 EuroWestNile and AGL2011-13634-E

Keywords: West Nile virus, Usutu virus, Bagaza virus, flavivirus, emerging diseases, Europe.

P-203

Antigen and antibody detection tools for West Nile virus

Belén Rebollo*[1], Javier Sarraseca[1], Ana Camuñas[1], Elena Soria[1], Carmina Gallardo[2], Miguel Ángel Jiménez Clavero[2], Ana Moreno[5], Paolo Cordioli[5], Ángel Venteo[1], Antonio Sanz[1], María José Rodríguez[1]

(1) Departamento de Investigación y Desarrollo. INGENASA. Madrid (2) Departamento de Virología. Centro de Investigación de Seguridad Animal (CISA) INIA. Valdeolmos, Madrid. (5) Departamento de Virología. IZSLER. Brescia, Italy.

West Nile Virus (WNV) is a member of Flavivirus genus of the Flaviviridae family and belongs to Japanese encephalitis serocomplex which includes Usutu virus. At present 9 lineages (L1 to L9) have been described and like the other members of the family is a zoonotic virus.WNV infection is one of the most widespread arboviral infections. Its transmission cycle involves mosquito-vectors (mainly *Culex spp.*) and birds as amplifying reservoirs, but a wide variety of vertebrate species, particularly equines and humans, are also susceptible to infection.The WNV genome consists of a single stranded positive-sense RNA molecule which encodes three structural proteins (capsid (C); premembrane (prM); and envelope (E)) and seven non-structural proteins (NS1, NS2A/B, NS3, NS4A/B, NS5). In the last 15 years the virus has spread quickly in many parts of the world. There are vaccines only for veterinary use (horses) and a differential diagnosis is essential due to the cross-reactivity between the flaviviruses.The aim of this work was the development of antigen and antibody detection tools to study and control the outbreaks of WNV. For this purpose we have expressed NS1 and E protein of WNV ,L1 and L2, and of Usutu virus. The WNV recombinant proteins were used to obtain a panel of Monoclonal Antibodies (Mabs).The reactivity of these Mabs against different Flaviviruses (including the Tick Borne Encephalitis Virus, probably the most important Flavivirus in Europe) was determined. In addition, a different panel of Mabs obtained by immunization of mice with Usutu virus from infected cells was characterized by their reactivity with the recombinant proteins. A double antibody sandwich ELISA able to differentiate between WNV and other

Flavivirus (Usutu) was developed using the NS1 WNV specific Mabs .On the other hand an IgM capture ELISA was developed using two Mabs: one specific for domain III of WNV and other specific for horse IgM. The serologic response in 5 vaccinated horses (2 doses of Pfizer WNV inactivated vaccine) was analyzed with this IgM ELISA in comparison with the response of infected animals. The results showed that the IgM response is similar in both groups suggesting that the presence of specific IgM is not indicative of infection. DIVA assays using NS1 protein are currently evaluated.Acknowledgments-
The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/ 2010-2013) under grant agreement nº 2613911

Keywords: flavivirus, West Nile, Usutu, tick borne, diagnosis.

P-204

Rescue of a foot-and-mouth disease virus (FMDV) mutant carrying a deletion in the 3'non-coding region by RNA trans-complementation

Miguel R. Rodríguez Pulido*[1], Mónica Gutiérrez Rivas[2], Francisco Sobrino[1], Margarita Sáiz[1]

(1) Departamento de Virología y Microbiología. CBMSO (CSIC-UAM). Madrid (2) Centro Nacional de Microbiología. Instituto de Salud Carlos III . Madrid.

The FMDV viral genome consists of a single ssRNA(+) molecule about 8.5 Kb in length, with a viral protein VPg covalently linked to the 5' end and a poly(A) tract at the 3' end. As a member of the *Picornaviridae* family, the viral RNA contains a single ORF flanked by two highly structured 5' and 3' non-coding regions (NCRs) enclosing motifs and structures involved in replication and translation, which are critical for FMDV infectivity.

In previous work, we have shown the involvement on viral replication of the two predicted stem-loop (SL) structures composing the 3'NCR, SL1 and SL2, respectively. Deletion of the SL1 in the 3'NCR generated viruses with a reduced growth capacity and down-regulated replication associated to a defect in negative-strand RNA synthesis, while deletion of SL2 was lethal for viral infectivity. Further, several mutants were generated on the DSL1 genotype bearing additional substitutions or deletions with the aim to define the minimal requirements in the SL2 structure for viral replication. Only one (S3 mutant) out of the five SL1 mutants bearing deletions could be recovered after transfection in porcine IBRS-2 cells. This S3 mutant genotype, carried two substitutions in the base of the loop and reverted to wt sequence after a first passage on cell culture. In order to test the putative rescue of DSL2 virus in the presence of the SL2 provided in trans, we performed co-transfection experiments combining the SL2 and SL1-S3 FMDV genotypes. Surprisingly, only DSL2 virus was recovered at late times after co-transfection of IBRS-2 cells. Moreover, the non-infectious DSL2 genotype could also be rescued when co-transfection experiments were carried out using a short RNA transcript corresponding to the SL2 enclosing the poly(A) tail instead of the complete SL1-S3 genome. The resulting SL2 virus showed a small-plaque phenotype that was maintained, to-

gether with the input SL2 sequence, at least after six passages on IBRS-2 cells. The involvement of the structural motifs in the FMDV 3´NCR, as well as other viral factors, in the SL2 genotype rescue will be discussed.

Keywords: foot-and-mouth disease virus; nonconding region; viral replication.

P-205

The VP2 protein of infectious pancreatic necrosis virus (IPNV) modulates its virulence and immunogenicity in Atlantic salmon (*Salmo salar L*)

Sylvia Patricia Rodríguez Saint-Jean*[1], Alex Romero[2], Natalia Andrea Ballesteros Benavides[1], Mónica Monrás[2], César Ortega[3], Ricardo Enríquez[2], Sara Isabel Pérez Prieto[1]

(1) Departamento de Microbiología Molecular. Centro de Investigaciones Biológicas CSIC. Madrid (2) Instituto de Patología Animal. Facultad de Veterinaria. Universidad Austral Chile. Valdivia, Chile (3) Centro de Investigación y Estudios de Salud Animal. Fac Veterinaria. Universidad Autónoma de Estado de México. Toluca, México.

IPNV is the aetiological agent of an acute and widespread disease that causes high mortality in fry and juvenile salmonid fish, as well as in smolts after their transfer to seawater. IPNV is a member of the Birnaviridae family, a group of double stranded non-enveloped RNA viruses that are 60nm in diameter. The IPNV genome is comprised of two parts, segment A, encoding the VP2, VP3, VP4 and VP5 proteins, while segment B codes for the RNA-dependent RNA polymerase, VP1. VP2 is the largest protein in this virus, to which most of the neutralizing epitopes of IPNV map. We have explored the ability of the VP2 protein of IPNV to elicit an immune response when an expression plasmid containing the gene is injected intramuscularly into salmon, analysing the transcription and kinetics of a group of selected genes in the kidney of these fish. The antiviral genes IFN- and Mx, the anti-inflammatory interleukin IL10, and the inflammatory IL 8, 11 and 12 genes were considered, as well as other genes encoding specific elements of the immune response, such as interferon- (a Th1 marker) and the membrane bound immunoglobulin isotype M (mIgM) as a marker of humoral activity. VP2 induced the expression of genes related to both innate and specific immunity, and their expression was modulated similarly in time course experiments. Peak expression of all the genes was reached on day 7 post vaccination and the maximum titre of anti-IPNV neutralizing antibodies was detected on day 15. The recovery and quantification of virus from VP2-stimulated and control fish provided evidence of some degree of protection. Moreover, the relevance of the VP2 gene in the virulence of IPNV was evident in experiments "*in vitro*" with small interfering RNAs (siRNAs). Three siRNAs were designed to silence VP2 expression by RNA interference and they were tested in BF-2 cells. Transfection of these siRNAs re-

duced the production of IPNV particles to a different extent, while the corresponding controls did not affect the particles recovered in supernatants from virally infected cells. Thus, these siRNAs specifically inhibited the expression of the viral target gene, reducing the corresponding viral load. In summary, the VP2-capsid plays a role in the immunogenicity and virulence of IPNV, providing a target antigen for the development of new IPNV vaccines.

This work was supported by grant AGL2010-18454 from MINECO, Spain.

Keywords: fish virus, Birnavirus, IPNV, VP2 protein.

P-206

Identification of immunodominant epitopes on the RVFV nucleoprotein defined by monoclonal antibodies

Gema Lorenzo*[1], Elena López[1], Alejandro Brun[1]

[1] Centro de Investigación de Sanidad Animal (CISA). INIA. Valdeolmos, Madrid.

Rift Valley fever (RVF) is a viral zoonosis that affects domestic ruminants as well as humans and is transmitted mostly by *Aedes sp* and *Culex* mosquitoes. Infection with RVF virus (RVFV) causes abortion of pregnant animals along with a high neonatal mortality in lambs and calves. In humans, it usually manifests itself with influenza-like symptoms, but occasionally leads to more serious complications like haemorrhagical fevers, encephalitis and retinitis with high morbidity and mortality. This virus is endemic in Africa and spread outside the continent to Arabian Peninsula, Madagascar and Comoros archipelago. Recently, outbreaks have occurred more often and the human cases have increased in morbidity and death. Furthermore, the presence of numerous species of potentially competent mosquito vectors in Europe, the global changes in climate, travel and trade confirm the potential of this virus to emerge in other countries. Development of more effective methods for RVFV outbreak prevention and control remains a global health priority. Serological diagnosis assays are based in the antibodies detection against the viral nucleoprotein. N protein is the most abundant component of the virion and is highly immunogenic producing high titers of anti-N antibodies in the host. The role of these antibodies is still unknown. In this work, using polyclonal and monoclonal antibodies, we were aimed to map immunodominant epitopes on the N protein. Expression of truncated forms of a recombinant RVFV nucleoprotein allowed mapping an immunodominant region in the first amino terminal third of the N protein. The location of the epitope(s) involved in antibody binding was more precisely determined using both a phage display library and a peptide array approaches. Two domains in a region comprised between amino acids 60 to 90 were identified. Several N protein mutants were generated either by deletion of each or both domains as well as by substitution of specific amino acid residues. Anti-RVFV sera and mAbs anti-N showed differential reactivity against these mutants, allowing the fine mapping of critical residues influencing the im-

munogenicity of the protein. The immune relevance of some of the identified epitopes in terms of induced humoral responses was also investigated upon immunization of mice.

Keywords: emerging disease, zoonosis, mosquitoes vector, epitope mapping.

P-207

Molecular epidemiology of Peruvian DENV-3 isolated in 2009 and 2010

Nancy L. Mayo*[1], Enrique W Mamani[3], Francisca Molero[2], Antonio Tenorio[2], Leticia Franco[2]

(1) Máster de Virología. Universidad Complutense de Madrid. (2) Laboratorio de Arbovirus y Enfermedades víricas importadas. Instituto de Salud Carlos III. Madrid. (3) Departamento de Virología. Instituto Nacional de Salud. Lima, Perú.

Dengue fever (DF) and its more severe form,Dengue Hemorrhagic Fever (DHF) is caused by dengue virus (DENV) ,that belongs to the family *Flaviviridae*. It is transmitted by *Aedes* mosquitoes and human acts as virus reservoir. WHO estimates that 50-100 million infections occur annually in tropical and subtropical regions and that 2.5 million people are at risk. It is endemic in America, Asia and Africa and indigenous outbreaks have occurred also in Europe in the recent years. All four known DENV (DENV-1 to 4) are circulating in the Americas. In this continent the incidence of dengue has increased in the last year to reach over 1.6 million of cases in 2010. To this figures, the Andean region contributed with 300,575 cases, whereas Peru reported 18,688 cases in the same year.

In Peru, dengue is a public health concern and the 4 serotypes coexist in the same area. In 2001, a dengue outbreak occurred in three cities of the North and the Amazon areas when sever e cases and deaths were reported. The responsibility for this largest outbreak was attributed to the introduction of a new genotype of DENV-3.

DENV-3 are classified into four genotypes I, II, III and IV. In 1989 an outbreak of DHF in Sri Lanka was correlated with the introduction of a new variant of DENV-3, designed genotype III or Sri Lanka or more recently named Indian/American. This strain spread from the Indian subcontinent to the world. In Latin America was introduced by Nicaragua and Panama in 1.994 and since then spread throughout South America and the Caribbean by partnering with the appearance of DHF. In the recent years, also was documented the circulation in Colombia of genotype I (Asian-pacific).

The aim of this work is to genotyping Peruvian strains of DENV-3 collected in 2009 and 2010.

We analyzed a total of 42 samples came from 12 departments (provinces). As result of first screening by Nested we found 4 DENV-3 from 2 departments. Then we proceeded to sequencing the complete envelope gene. Phylogenetic analysis revealed that all samples belong to genotype III of DENV-3, but samples of Lima (coastal area) had 99% of identity with Venezuelan and Colombian strain (2001-2003). However strains circulated in

Madre de Dios (Amazonic sylvatic area) had 100% identity with Brazilian strain(2006-2007). In conclusion, we can infer that by 2010 in Peru is still circulating DENV3 genotype III and has been responsible for outbreaks in these last years.

Keywords: flavivirus, dengue, DENV-3 phylogenetic analysis

P-208

A case of imported dengue from Madeira outbreak: an epidemiological threat?

Montserrat Montes de Oca[2], Leticia Franco[3], Inmaculada Guerrero Lozano[1], Clotilde Fernández Gutiérrez del Álamo[1], Fernando de Ory[3], Manuel Rodríguez Iglesias*[1]

(1) Department of Clinical Microbiology. Puerta del Mar Univ Hosp. Cádiz (2) Department of Internal Medicine. Puerta del Mar Univ Hosp. Cádiz (3) Laboratory of Serology. National Center for Microbiology. Majadahonda, Madrid.

An outbreak of dengue fever in Madeira Island was reported in 2012, and between early October 2012 and the beginning of 2013, 2,144 autochthonous dengue virus infections were reported. The 2012 Madeiran epidemic has had a great impact on local residents, but has also affected visitors of the island. The latest available data from February 2013 reports that 78 visitors from European countries got infected in Madeira. We describe a case of dengue in a cruise crew arrived at the port of Cadiz from Madeira and is one of two imported cases registered in Spain.

A 20 year-old womanwhich is part of the crew of a cruise ship, developed febrile illness with malaise, anorexia, arthralgias and myalgias five days after arrive to Madeira. The clinical course was worsening with nausea and vomiting, and five days later with the appearance of a non petechial erythematous rash in upper and lower limbs and spread to abdomen are advised. On 11 November, 2012 she arrived at the port of Cadiz, and was admitted to the hospital. Ten days before the ship had made a stop in Madeira. The first laboratory findings in the hospital showed thrombocytopenia ($112x10^9$/L), leucopenia ($3.0x10^9$/L) and elevated transaminases (ALT: 442U/L and AST: 467 U/L). Serum samples were tested by ELISA for IgG and IgM, by using commercial reagents (Panbio, Brisbane, Australia) . The serum sample (day 10 post onset) was positive for dengue virus specific IgM with a indx of 78.40 (indeterminate = 9-11) and IgG with a index of 181.00 (indeterminate = 9-11). IgM detection was confirmed with a confirmatory assay, in presence and in absence of antigen. Two days later, the platelet decrease to $60x10^9$/L although without hemorrhagic complications. The evolution was favourable andthe patient was discharged three days later.

Similarly to Madeira, it has been suggested that island locations that are major tourist attractions may be at elevated risk for dengue introduction via travellers if suitable vectors are available. According to ECDC VBORNET mosquito maps no information is currently available on competent dengue vectors, for

some locations situated relatively closely to Madeira, such the Canary Islands. An internationally coordinated response is indeed needed in view of the challenges posed not only by this outbreak, but also by outbreaks in other islands of similar size that share characteristics like a tourism-dependent economy and vulnerability to vector-borne diseases.

Keywords: dengue, Madeira, outbreak.

P-209

A genetic survey of Crimean-Congo hemorrhagic fever virus in ticks from Spain in 2011

Ana Negredo*[(1)], Fátima Lasala[(1)], Eva Ramírea de Arellano[(1)], María Dolores Fernández[(1-2)], Juan Manuel Luque[(1)], Miguel Ángel Habela[(3)], Agustín Estrada Peña[(4)], Antonio Tenorio[(1)]

(1) Laboratorio de Arbovirus y Enfermedades Víricas Importadas. Centro Nacional de Microbiología, Instituto de Salud Carlos III. Madrid (2) European Public Health Microbiology Training Fellow (EUPHEM. European Centre for Disease Prevention and Control (ECDC). Solna, Sweden (3) Departamento de Parasitología y Enfermedades Parasitarias. Facultad de Veterinaria, Universidad de Extremadura. Cáceres (4) Departamento de Parasitología. Facultad de Veterinaria, Universidad de Zaragoza.

Crimean-Congo hemorrhagic Fever virus (CCHFV) is a zoonotic virus that causes outbreaks of hemorrhagic fever in human with a fatality rate up to 30%. The viral cycle is maintained by ticks of *Hyalomma* spp., serving as vectors and reservoirs of the virus and responsible for the worldwide distribution of the CCHFV. Viremia or antibody production has been demonstrated in vertebral animals that suffer an asymptomatic infection. Transmission of CCHFV in human occurs through tick bites, direct contact with blood or tissues of infected livestock, person to person contact or by nosocomial infection. It is considered a biological high risk virus without available therapeutic tools.

CCHFV belongs to the *Nairovirus* genus, family *Bunyaviridae*. It is a negative-stranded, enveloped RNA virus having a tripartite genome consisting of S, M and L segments, which encode the viral nucleocapsid, glycoprotein precursor and polymerase proteins, respectively. Unlike other viruses with a unique ecological niche, this virus has evolved separately in different geographical areas, giving rise to 20, 31 and 22% of nucleotide variability among virus S, M and L genome segments, respectively (Deyde *et al* 2006). The segment phylogenetic trees topologies document seven distinct lineages, usually coherent between the three segments, although segment re-assortment (Hewson *et al* 2004) and recombination events also occur (Chare *et al* 2003).

In Spain, CCHFV genome was detected in ticks collected from red deer in Cáceres in 2010 (Estrada-Peña *et al* 2011). The finding was confirmed using a different target at the S segment, and the analysis of the amplified fragment suggested the presence of an African related strain included in Genotype III. One year later, 500 ticks were collected from different areas of Cáceres, Toledo and Huesca in order to

check its temporal persistence and geographical distribution.

Results: 221 ticks collected in 2011 were analyzed for the presence of CCHF genome using a Nested-RT-PCR designed at S genome segment. 11 positive results were obtained in ticks collected in Cáceres and ticks from Toledo and Huesca resulted negative. Nucleotide sequence analysis revealed that all viral genomes belong to genotype III.

Conclusion: The present investigation shows the persistence of CCHFV in ticks in the geographical region of Cáceres. It is necessary advance in future investigation to know the geographic extension, biological cycle and the origin of CCHFV in Spain.

Keywords: CCHFV, ticks, RT-PCR.

P-210

Standardization of experimental infection protocol for West Nile virus infection in a mouse model

Elisa Pérez Ramírez*[1], Francisco Llorente[1], Javier del Amo[1], Miguel Ángel Jiménez Clavero[1]

(1) Centro de Investigación en Sanidad Animal (CISA. INIA). Valdeolmos, Madrid.

Rodent models for West Nile virus infection have been used extensively (Granwehr BP et al, 2004) because they are susceptible to WNV infection and develop severe neurological symptoms reminiscent of those observed in human WNV neuroinvasive disease. However, large variations in susceptibility to the virus can be found among studies (Beasley DWC et al, 2002; Cordoba L et al, 2007), which suggests that WNV susceptibility in mice is affected by several factors such as genetic background and mouse age (being younger mice the most susceptible). Different pathogenicity results are also common among experimental studies using same WNV strain and type of mice. This variation could be related to methodological differences during titration of viruses, preparation of inocula and the inoculation route. Standardization studies are needed to improve reproducibility of the results in rodent model of WNV disease. The aim of this study was to standardize an experimental infection protocol that allows maximum accuracy and reproducibility of results during experimental infection trials. Different factors were studied to assess their influence on pathogenicity results, such as the effect of time from thawing of viruses to inoculation of mice and the effect of albumin addition to the diluent used in titration and inoculation protocols. Parallel titration of viruses that will be inoculated and the use of PBS+0.2% albumin as diluent during titration techniques and preparation of inocula proved to be crucial factors enabling the best reproducibility and minimizing variation in pathogenicity results between experiments.

Granwehr BP, Lillibridge KM, Higgs S, Mason PW, Aronson Jf, Campbellga, et al. (2004) West Nile virus: where are we now? Lancet Infect Dis 4(9):547-56
Beasley DW, Li L, SudermanMT, Barrett AD. (2002) Mouse neuroinvasive phenotype of West Nile virus

strains varies depending upon virus genotype. Virology 296(1):17-23
Cordoba L, Escribano-Romero E, Garmendia A, Saiz JC (2007) Pregnancy increases the risk of mortality in West Nile virus-infected mice. J Gen Virol 88(2):476-80

Keywords: West Nile virus, mouse model, experimental infection.

P-211

The human respiratory syncytial virus P protein through different phosphorylations coordinates viral protein interactions essential for the viral RNA synthesis

Nieves Villanueva*[1], Ana Asenjo [1], Marisa Navarro[1]

[1] Unidad de Replicación Viral. Centro Nacional de Microbiologia. Instituto de Salud Carlos III. Madrid.

The human respiratory syncytial virus (HRSV), a pneumovirus of the paramyxovirus family, is the main cause of acute lower tract respiratory infections affecting to babies and toddlers and aged or inmunocompromised adults. It is estimated 34 million infections in children under 5 years of age and 199000 pediatric deaths annually worldwide (1). Beside a wide research on HRSV molecular biology carried out in the last fifty years there is not vaccine and the fact that the primoinfection should not be able to produce a protective for living immune response suggests that other reactives such antiviral compounds among others are needed to control human respiratory syncytial infections.

To develop these compounds and according to the parasite nature of the viruses, it is important to know how the viral protein work during the viral growth cycle in order to delimitate steps of the process that tagged differentially the viral metabolism. In the paramyxovirus family, the way in which viral RNA synthesis occurs is completely different of the cellular RNAs generation process and it is carried out by a RNA dependent polymerase (RdRp) virally encoded .The template is genomic viral RNA (minus or plus polarity) bound to the N protein (never naked RNA)and the RdRp is a complex of the L protein or polymerase and P protein.

The P protein is a phosphoprotein in which different residues are modified with different turnover. Our work has been focused on the determination of P protein modified residues, and in the identification of the cellular protein kinases and phosphatases involved in P protein phosphorylations. The functions that P protein developed in viral RNA synthesis according to its phosphorylation state should be discussed.

1.Nair et al., 2010. Lancet 375:1545–1555

Keywords: human respiratory syncytial virus, P protein, phosphorylation, protein kinases and phosphatase.

P-212

Strain specific autophagic response in cells infected with West Nile and Usutu flaviviruses

Ana B. Blázquez*[1], Estela Escribano Romero[1], Juan Carlos Sáiz[1], Miguel A. Martin Acebes[1]

(1) Departamento de Biotecnología. Inst. Nac. Investigaciones Agrarias. Madrid.

Autophagy plays an important role on different aspects of viral infections, and therefore influences viral pathogenesis. Regarding the *Flaviviridae*, autophagy has been associated to different aspects of the replication and pathogenicity of some members of this virus family. In the case of the mosquito-borne flavivirus West Nile virus (WNV), the induction or not of autophagy remains contentious. One recent report pointed that WNV infection induced an autophagic response, whereas another suggested that the autophagic pathway was not upregulated in WNV-infected cells. In this report we have analyzed the induction of autophagy following infection with different strains of WNV that varied temporally, geographically, and in their degree of neurovirulence, and with Usutu virus (USUV), a closely related mosquito-borne flavivirus in which the involvement of the autophagic machinery during its replication has not been previously explored. The ability to provoke an autophagic response on infected cells by the different viral strains assayed was compared to different aspects related to viral pathogenicity as cell killing ability, induction of apoptosis, induction of unfolded protein response, and neurovirulence, providing new insights into the involvement of the autophagic pathway during WNV and USUV infection.

All viruses tested induced the unfolded protein response in infected cells, and, except one, stimulated the autophagic process, which was demonstrated by an increase of cytoplasmic aggregation of microtubule-associated protein 1 light chain 3 (LC3), a marker of autophagosome formation. In addition to this, an increase on the lipidated form of LC3 was noticed following infection with USUV or different WNV strains. Noticeably, the only exception was observed after infection with the highly pathogenic WNV isolate responsible for the outbreak of encephalitis in New York in 1999 (WNV-NY99) that, conversely, did not display autophagic features and showed a different behaviour to USUV and the other WNV strains analyzed. Implications of the role of autophagy for infection and pathogenesis of WNV and USUV are discussed.

Keywords: autophagy, LC3, replication, West Nile Virus, Usutu virus

P-213

Cellular autophagy machinery is not required for torovirus replication

Ginés Ávila Pérez*[1], Susana Plazuelo Calvo[1], Dolores Rodríguez Aguirre[1]

(1) Departamento de Biología Molecular y Celular. Centro Nacional de Biotecnología (CSIC). Madrid.

The autophagy is a conserved eukaryotic mechanism that mediates the removal of long-lived cytoplasmic macromolecules and damaged organelles via a lysosomal degradative pathway. Recent studies suggest that some positive-stranded RNA viruses exploit this pathway to facilitate their own replication. In this study, we investigated the role of autophagy in equine torovirus replication. Toroviruses (*Nidovirales* order) are gastroenteritis causing agents that infect different animal species and humans. In this study we demonstrate that equine torovirus Berne virus (BEV), the prototype member of the torovirus genus, induces autophagy in infected cells at late times post-infection. BEV infection causes an increase in the conversion of LC3I to LC3II that is associated with autophagosome formation. This result was confirmed by the generation of equine cells that express GFP-LC3 that serves as a marker of autophagosome formation. BEV induced the accumulation of GFP-LC3 foci in the infected cells, as it occurs after induction of autophagy by nutrient deprivation. However, the replication of BEV was not affected in autophagy deficient cells lacking Beclin-1, a protein critical for autophagosome formation. We have generated equine cells that produced short harping RNA or interfering RNA to silence equine*Beclin-1* gene. Depletion of Beclin-1 resulted in a reduction in the autophagosome formation, but BEV replication, as determined by virus titration, was not affected in this cells line. Similarly, induction of autophagy by nutrient deprivation does not produce changes in the viral protein accumulation or in the viral titer. Taken together, our results suggest that the autophagy pathway is activated during BEV infection at late time post-infection, but this activation is not required for the viral replication.

Keywords: torovirus, autophagy, autophagosome.

P-214

Ultrastructural characterization of membranous torovirus replication factories

Ginés Ávila Pérez*[1,2], Sylvia Gutiérrez Erlandsson[2], Mª Teresa Rejas Marco[3], Dolores Rodríguez Aguirre[1]

(1) Departamento de Biología Molecular y Celular. Centro Nacional de Biotecnología CSIC. Madrid (2) Servicio de Microscopía Confocal. Centro Nacional de Biotecnología-CSIC. Madrid (3) Electron Microscopy Facility. Centro de Biología Molecular Severo Ochoa CSIC. Madrid.

The replication complexes of all positive stranded RNA viruses are associated with modified host cell membranes. We previously described the presence of double membrane vesicles (DMVs) in the cytoplasm of cells infected with the equine torovirus Berne virus (BEV), the prototype member of *Torovirus* genus (*Coronaviridae* Family, *Nidovirales* Order), which accumulated in their interior double stranded RNA (dsRNA), an intermediate of viral replication. However, little is known about the biogenesis of these DMVs and their dynamics during the infection. Using confocal microscopy and transmission electron microscopy we examined the distribution of viral replicase proteins and the ultrastructural changes that occur during the BEV infection. By confocal microscopy we observed that the replicase proteins colocalize during the infection, giving a punctuated signal at early times, and being aggregated in patches located close to the nucleous at later times postinfection. A similar pattern was observed when nascent RNA was analyzed, however, an apparent differential distribution was observed for dsRNA. At the ultrastructural level we found that DMVs form a reticulovesicular network (RVN) that resembles those previously described in the related viruses severe acute respiratory syndrome coronavirus (SARS-CoV), and the equine arteritis virus (EAV), where the outer membranes of the DMVs are interconnected with each other and with the ER. In accordance with the changes observed by confocal microscopy in the localization of replicase proteins through the infection, we observed small RVNs (factories) dispersed in the cytoplasm at early times, which became more complex, and were relocalized to the perinuclear area at late times postinfection. Also, as in SARS-CoV, the RNV induced by BEV is formed by DMVs and convoluted membranes. The convoluted membranes were immunolabeled with antibodies directed against replicase proteins, but the DMVs interior was devoid of signal. Then, as observed by confocal microscopy the replicase proteins did not colocalize with dsRNA. This apparent conflict was resolved with a three-dimensional reconstruction of confocal images where the dsRNA is shown inside of cavities formed by the replicase proteins. Our results with BEV show a high similarity with those obtained with other related viruses in the *Nidovirales*order. Taken together, these date indicate that the nidoviruses use a similar strategy to establish their membranous replication factories.

Keywords: Torovirus, reticulovesicular network, DMVs, convoluted membrane.

P-215

A sensitive method to quantify replicative forms of circular DNA viruses

Edgar A. Rodríguez Negrete[1], Sonia Sánchez Campos[1], Jesús Navas Castillo[1], Enrique Moriones[1], Eduardo R. Bejarano[1], Ana Grande-Pérez*[1]

(1) Departamento de Genética, Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" (IHSM-UMA-CSIC). Universidad de Málaga. Consejo Superior de Investigaciones Científicas. Málaga.

Geminiviruses are plant viruses with circular single-stranded DNA (ssDNA) genomes transmitted by insects that include emerging and economically significant viruses. Although real-time PCR diagnostic protocols for their detection are available, none of them are able to discriminate between the two strands generated during an infection: the viral strand (VS) that encodes the coat protein, CP, and a movement-like protein, V2, and is encapsidated within virions; and the complementary-sense strand (CS) that codes for proteins involved in replication (Rep and C3), transcription (C2) and symptom development (C4), and is used as template to generate more viral strands by a rolling circle mechanism. Here we describe a two-step real-time PCR protocol to quantify the amount of VS and CS as well as how many of those strands are arranged as single or double strand (dsDNA). The method was set and tested on synthesized VS and CS circular molecules of the begomoviruses Tomato yellow leaf curl Sardinia virus (TYLCSV) and Tomato yellow leaf curl virus (TYLCV), both involved in Tomato yellow leaf curl disease. Then the amount of VS and CS was determined in systemic infections of TYLCSV and TYLCV in tomato and *Nicotiana benthamiana* plants. The results show that the ratio VS/CS is not constant throughout the time of infection and depends on the combination virus-host. In tomato, the increment of ssDNA measured at 7 and 42 days post-infection in both viruses was due mostly to synthesis of VS. In both viruses more than 98% of their CS is arranged as dsDNA, while VS is disposed both as ssDNA and dsDNA. We also measured the amount of ssDNA of both polarities in *N. benthamiana* leaves agroinfiltrated with TYLCSV C2, C3, C4 and V2 mutants. The results show that C2, C4 and V2 mutants accumulate similar amounts of DNA, both as ssDNA and dsDNA, and have VS/CS ratios comparable to the wild type TYLCSV. However, the C3 mutant presents reduced amounts of all species of viral DNA. The protocol described here is a significant improvement of the techniques in use to quantify circular ssDNA and can help to understand in detail the molecular scenario during replication of any viruses whose genome is made of circular DNA.

Keywords: viral-sense ssDNA, complementary-sense ssDNA, two-step qPCR, begomovirus, TYLCSV, TYLCV

P-216

RNPs function as a transcriptional unit independent from the capsid in the dsRNA virus IBDV

Romy M. Dalton*[1], José Francisco Rodriguez[1]

(1) Departamento de Biología Molecular y Celular. Centro Nacional de Biotecnología. Madrid.

Viruses with an RNA genome are divided into classes depending on the transcription and replication strategy used to escape the cell defense mechanisms induced by RNA duplex. Positive-stranded ssRNA viruses limit RNA synthesis to low levels so dsRNA regions are scarcely accumulated. Negative-stranded ssRNA viruses shield de genomic RNA with a nucleoprotein forming ribonu-

cleoproteins (RNPs). And dsRNA viruses normally hide the genome inside a transcriptional core T=2 capsid that contains the machinery necessary for transcription and that is covered by one or two T=13 capsid shells. New transcripts are extruded into the cell cytoplasm through the capsid pores for translation. However, Birnavirus is the only dsRNA family described with a single-shelled T=13 capsid and the genome bounded to a nucleoprotein (VP3) and the RNA-dependent RNA polymerase (VPg), forming RNPs. We developed a cell based RNP generation system to elucidate the single T=13 capsid of IBDV, a member of this family, could function as a transcriptional core or if RNPs are rather released into the host cell and work as the independent transcription machinery. Using this system, as well as analyzing IBDV infections, we demonstrate that IBDV is the only dsRNA virus described up to date lacking a transcriptional core and which releases the genome into the cytoplasm in the form of RNPs, which constitute the viral transcription and replication machinery.

Keywords: IBDV, transcription, replication.

P-217

Identification of myxobacterial metabolites affecting mammalian P-body formation with overlapping antiviral activities

Javier P Martínez*[1], Gemma Pérez-Vilaró[1], Nicoletta Scheller[1], Yazh Muthukumar[2], Tatjana Hirsch[2], Ronald Frank[2], Florenz Sasse[2], Andreas Meyerhans[1,3], Juana Díez[1]

(1) Department of Experimental and Health Sciences. Universitat Pompeu Fabra. Barcelona (2) Department of Chemical Biology. Helmholtz Centre for Infection Research. Braunschweig, Germany. (3) Institució Catalana de Recerca i Estudis Avançat (ICREA). Barcelona.

Processing bodies (P-bodies) are highly conserved and dynamic mRNP structures that play a central role in the control of posttranscripitional gene expression. Remarkably, P-body components have also been shown to play a role in cancer and in viral infections. Therefore, P-bodies are not only interesting for the study of mRNA regulation, but have emerged as an appealing target for drug discovery. To date, analysis of P-body structure and function in higher eukaryotes involve the depletion of P-body components by siRNA technologies. While these tools are of great help in understanding mRNA regulation, a complementary approach that identifies and uses small chemicals affecting these mRNP structures might help reveal not only uncharacterized P-body features but also provide novel antiviral drugs. Here we established an automated cell-based assay to screen for molecules acting directly or indirectly on P-body formation. The platform was used to inves-

tigate the effects on P-bodies of a unique library of around 150 natural compounds derived from myxobacteria, one of the top microbial producers of secondary metabolites that have the property to target diverse eukaryotic processes. Overall, and with a baseline of no more than 30% reduction in cell viability, almost 20% of the compounds screened showed significant activities against P-body formation. Interestingly, about one third of the hits have been described as anti-fungals and/or antimicrobials, including antivirals. The overlapping activities of the myxobacterial metabolites makes this group of bacteria a very important source of new therapeutics with putative broad-spectrum mode of action. For this, the development and application of high-throughput screening platforms like the one described here, are tools of upmost importance.

Keywords: P-bodies, screening, myxobacteria, broad-spectrum.

P-218

Construction and characterization of recombinant pseudorabies virus (PRV) by using BAC80 deficient in pac sequences

Laura Lerma*[1], Ana L Muñoz[1], Lourdes Varela[1], Mirela Dinu[1], Isabel Díez[1], Beatriz Martín[1], Ignacio Gadea[2], Enrique Tabarés[1]

(1) Dpto. Medicina Preventiva, Salud Pública y Microbiología. Facultad de Medicina. Universidad Autónoma de Madrid. (2) Servicio de Microbiología Médica. Fundación Jiménez Díaz. Madrid.

Pseudorabies Virus (PRV) is a member of the subfamily *alphaherpesvirinae,* and has all advantages of HSV-1 to be used in gene therapy strategies, besides to be a safer vector because is unable to cause disease in humans (Sawitzky 1997), eliminating the possibility of recombination between vector and the pre-existing latent virus in treated persons, moreover the pre-existing immunity is null (Prieto *et al.*, 2002; Oehmig *et al.*, 2004). Obviously, PRV can infect and transfer foreign genes to individual human cells. For all these reasons it could become a good candidate for use in gene therapy strategies (Prieto *et al.*, 2002; Boldogkoi and Nogradi 2003; Oehmig *et al.*, 2004).

The work presented here describes a different strategy for generation of PRV recombinant viruses, their characterization and biological properties of these viruses, which could be used in gene therapy and vaccine development. We generated the PRV-BAC80, which has only one copy of IE180 gene (the only immediately early gene) located in IR repeat region, the expression of this protein is tetracycline-regulated, and is deficient in pac1-pac2 regions. Expression recombinant viruses were selected by recombination with plasmids that contain viral pac sequences. In this work, PRV-BT80GF, PRV-HgD and PRV-HgDB viruses were obtained, which express EGFP, gD and a chimeric gDB of herpes simplex virus type 2 proteins, respectively. These recombinant viruses were characterized and their biological properties were also determined. In addition, PRV-BT80CEA and PRV-BT80TER viruses were constructed, which express a copy of IE180 under control of CEA and hTERT human promoters, respectively. These recombinant viruses were characterized and their biological properties were also determined.

References. Boldogkoi Z. and Nogradi A. (2003). Gene and cancer therapy-pseudorabies virus: a novel research and therapeutic tool? *Curr. Gene Ther.* 3: 155-182
Oehmig A., Fraefel C. and Breakefield X.O. (2004). Update on herpesviruses amplicon vectors. *Mol. Ther.* 10: 630-643
Prieto J., Solera J. and Tabares E. (2002). Development of new expression vector based on Pseudorabies virus amplicons: application to human insulin expression. *Virus Res.* 89: 123-129
Sawitzky D. (1997). Transmission, species specificity, and pathogenicity of Aujeszky's disease virus. *Arch. Virol. Suppl.* 13: 201-216

Keywords: herpesvirus, PRV, vectors, recombinant viruses.

P-219

Electrostatic repulsions at neutral pH underlie the weak thermal stability of foot-and-mouth disease virus, and guide the engineering of modified virions of increased stability for improved vaccines

Verónica Rincón Forero[1], Alicia Rodríguez Huete[1], Michiel M Harmsen[2], Mauricio García-Mateu*[1]

(1) Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), Universidad Autónoma de Madrid. (2) Central Veterinary Institute of Wageningen UR. Wageningen, Germany.

We are investigating the molecular basis of physical stability of virus particles in order to understand virus assembly, stability and dynamics, and also for bio-nanotechnological purposes including thermostable vaccines. One of our model systems is foot-and-mouth disease virus (FMDV), the causative agent of one of the economically most important animal diseases worldwide. In the present study we have investigated the molecular mechanism by which mutation A2065H in capsid protein VP2 exerts a greatly thermostabilizing effect on the virion against dissociation into pentameric subunits. The results have revealed the presence in the virion of coulombic repulsions between pentamers, even at neutral pH, which contribute to explain the low thermostability of FMDV and its empty capsid. Several acidic residues not far from residue A2065 contribute to this repulsion. Most likely, mutation A2065H stabilizes the virion because the additional positive charge introduced may partly neutralize some of the excess negative charge around, thus weakening the interpentameric repulsion. The discovery of this repulsive effect between pentamers at neutral pH allowed us to undertake a new rational protein engineering approach on FMDV that led to obtain four virus variants of increased thermostability. These engineered FMDVs constitute good candidates for development of thermostable vaccines against FMD based on virions or empty capsids.

Keywords: foot-and-mouth disease virus, virus particles, thermostable vaccines, electrostatic repulsion, thermal stability.

P-220

Comparative study of cellular modifications induced by adenovirus: wild type, packaging and maturation mutants

Gabriela N. Condezo Castro*[1], Marta del Álamo[1], Sara J. Flint[2], Miguel Chillón[3], Carmen San Martín[1]

[1] Departamento de Estructura de Macromoléculas. Centro Nacional de Biotecnología. Madrid [2] Department of Molecular Biology. Princeton University. New Jersey, USA [3] Departamento de Bioquímica y Biología Molecular. Universidad Autónoma de Barcelona.

The maximum viral titer of human adenovirus type 5 (Ad5) is obtained at 36hpi (hours post-infection). At this time of infection, Ad5 has induced several well-characterized cellular modifications. Ad5/FC31, an Ad5 mutant with two insertions (*attB*/*attP*- C31) flanking the packaging domain, has a delayed viral cycle, 20 hours longer than wt (wild type); however, its replication and protein expression are normal (Alba *et al*, 2005; Alba *et al*, 2007). Alba et al, 2011 observed that the delay is mainly affecting packaging of the viral genome. We are taking advantage of this alteration in the viral cycle to study adenovirus assembly within the cell. Using electron microscopy (EM), we have compared changes in the nuclear structure of cells infected with wt Ad5 or Ad5/FC31. Apart from the changes previously described in the bibliography, we observed a new structure specific for Ad5/FC31 that we called "speckled bodies" (SBs) due to their aspect at the electron microscope. SBs seem to contain subviral particles trapped in DNA-rich regions, and their size varies in range between 0.5 and 3 µm. Interestingly, SBs also appear in cells infected with *ts1*, a mutant defective not in packaging but in maturation. This observation suggests that packaging and maturation could be coupled during adenovirus assembly. To determine the composition of SBs, we are following viral DNA and DNA-packaging proteins (IVa2 and L1 52-55kDa) using bromodeoxyuridine or specific antibodies in immune-fluorescence assays. We have observed differences for the DNA distribution pattern between wt and both Ad5/FC31 and *ts1* mutants, but not for the proteins. In all cases, IVa2 and L1 52-55k form amorphous clusters and small rings (0.5-1.5µm). We show for the first time that both proteins appear in the same ring-shaped structures but not in all amorphous clusters. We are currently expanding the fluorescence study to EM for obtaining higher resolution localization information.

Alba *et al*. 2005. Gutless adenovirus: last-generation adenovirus for gene therapy. Gene Therapy 12,S18-S27

Alba *et al*. 2007. Differential amplification of adenovirus vectors by flanking the packaging signal wit *attB/attP*-PhiC31 sequences: implications for helper-dependent adenovirus production. Virology 367:51-58

Alba *et al*. 2011. Altering the Ad5 packaging domain affects the maturation of Ad particles. PLoS One 6(5):e19564

Keywords: adenovirus assembly, cellular modifications, packaging and maturation mutants.

P-221

Looking for adenovirus non-icosahedral components by cryo-electron tomography

Ana J. Pérez Berná*[1], Javier Chichón[1], José J. Fernández[1], Dennis Winkler[2], Juan Fontana[2], Jane Flint[3], José L. Carrascosa[1], Alasdair C. Steven[2], Carmen San Martin[1]

[1] Department of Macromolecular Structures. Centro Nacional de Biotecnología - CSIC. Madrid [2] Laboratory of Structural Biology Research. National Institutes of Health (NIH). Bethesda, USA [3] Molecular Biology Faculty . Princeton University. USA.

Adenovirus has a non-enveloped icosahedral capsid enclosing a 35 kbp linear dsDNA genome associated with ~25 MDa of DNA-binding proteins, making up a non-icosahedral core. Digestion of cores with staphylococcal nuclease, combined with EM analyses of viral disruption products and stoichiometric calculations had suggested a model where the viral DNA and core proteins are organized into approximately 180 nucleosome-like units termed "adenosomes" (1). Additionally, previous studies predicted that the icosahedral symmetry of the capsid would be broken at one of the 12 vertices, containing a portal structure (2). We are using cryo-electron tomography to visualize the non-icosahedral elements of adenovirus. This technique allows calculation of 3D maps of individual virions without averaging or enforcing the icosahedral symmetry (3). We have extracted, aligned and classified the vertex regions from 612 individual virus tomograms using maximum-likelihood subtomogram averaging methods (4). This procedure reveals that the vertices in each virion can be categorized in three groups, according to the relation between the icosahedral shell and the internal contents. In each viral particle, one vertex is in direct contact with the core, while the opposed vertex has a gap between shell and core, and the other 10 vertices present an intermediate situation. This observation may indicate the presence of additional proteins beneath one singular vertex (eg the packaging machinery), or an asymmetry in the distribution of the genome and accompanying proteins within the virion. Additionally, our analysis shows that each particle contains 150-180 discrete ellipsoidal densities asymmetrically distributed within the viral core. This is the first time that "adenosomes" have been directly observed in their natural environment.

1. Russell WC. 2009. *J Gen Virol* 90: 1-20
2. Christensen JB, Byrd SA, Walker AK, Strahler JR, Andrews PC, Imperiale MJ. 2008. *J Virol* 82: 9086-93
3. Grunewald K, Cyrklaff M. 2006. *Curr Opin Microbiol* 9: 437-42
4. Scheres SH, Melero R, Valle M, Carazo JM. 2009. *Structure* 17: 1563-72

Keywords: adenovirus, cryo-electron tomography.

P-222

Mechanical stability and reversible failure of vault particles

Aida Llauró* [1], Pablo Guerra[2], Nerea Irigoyen[3], José F. Rodríguez[4], Núria Verdaguer[2], Pedro J. de Pablo[1]

(1) Departamento de Física de la Materia Condensada. Universidad Autónoma de Madrid. (2) Institut de Biología Molecular de Barcelona. CSIC. (3) Division of Virology, Department of Pathology. University of Cambridge. UK (4) Centro Nacional de Biotecnología. CSIC. Madrid.

Vaults are the largest ribonucleoprotein particles found in eukaryotic cells, with an unclear cellular function and promising applications as drug delivery containers. In this paper we study the local stiffness of individual vaults and probe their structural stability with Atomic Force Microscopy under physiological conditions. Our data show that the barrel, the central part of the vault, governs both the stiffness and mechanical strength of these particles. In addition, we provoke single protein fractures in the barrel shell and monitor their temporal evolution. Our high-resolution AFM topographies show that these fractures occur along the contacts between two major vault proteins and disappear over time, thus removing any mark of the previous rupture. This unprecedented systematic self-healing mechanism, which may enable these particles to reversibly adapt to certain geometric constraints, might help vaults safely pass through the nuclear pore complex.

Keywords: atomic force microscopy, indentation, fracture, self-healing, nanoshell

P-223

Mapping *in vitro* physical properties of intact and disrupted virions at high resolution using multi-harmonic atomic force microscopy

Mercedes Hernando*[1], Alexander Cartajena [2], José López Carrascosa[3], Pedro José de Pablo[1], Arvind Raman[4]

(1) Departamento de Física de la Materia Condensada. Univesidad Autónoma de Madrid. (2) School of Mechanical Engineering . Purdue University. West Lafayette, USA (3) Departamento de Estructura de Macromoléculas. Centro Nacional de Biotecnología. Madrid (4) School of Mechanical Engineering and the Birck Nanotechnology Center. Purdue University

Viruses are striking examples of macromolecular nano-machines which carry out complex functions with minimalistic structure. Understanding the relationships between viral material properties (stiffness, charge density, adhesion, viscosity), structure (protein sub-units, genome, receptors, appendages), and functions (self-assembly, stability, disassembly, infection) is of significant importance in physical virology and nanomedicine application (1-2).

We present quantitative mapping of local quantitatively at nanometer resolution the local electromechanical force gradient, adhesion, and hydration layer viscosity *within* individual Bacertiophage 29 using the multi-harmonic atomic force microscopy technique under physiological condition. The technique significantly generalizes recent multi-harmonic theory and enables high-resolution *in vitro* quantitative mapping of multiple material (3).

High-resolution quantitative maps of bacteriophage 29 show that the material properties changes over the entire virion provoked by the local disruption of its shell, providing evidence of bacteriophage despressurization (4).

(1) Carrasco C, et al Proc. Natl. Acad. Sci. U. S. A., (2006), 103:13706-13711
(2) T. Douglas and M. Young, *Nature*, 1998, 393, 152-155
(3) Raman A, et al. (2011) Nature Nanotech 6: 809-814
(4) Hernando-Pérez, M et al., *Small*, 2012, 8, 2365

Keywords: AFM, bacteriophage phi29, physical virology, local quantitative maps.

P-224

Investigating the *in vitro* self-assembly of empty capsids using computer simulations

Maria Aznar[1], David Reguera*[1]

[1] Departament de Física Fonamental. Universitat de Barcelona.

The self-assembly of the capsid is one of the most important steps in the replication cycle of a virus. Remarkably, the coat proteins of many viruses have the striking capability of self-assemble *in vitro* even in the absence of genetic material forming empty capsids that can be used for biomedical or nanotechnological applications. We have investigated the kinetics of assembly of empty capsids using computer simulations of simplified coarse-grained models. The results of the simulations provide new insights into the microscopic mechanisms of the assembly process, including the rate of capsid formation, the size of the critical nucleus or the dependence of the process on the protein concentration and assembly conditions. We will discuss these results as well as possible routes to interfere and control the assembly process with potential implications for the development of novel wide-spectrum strategies to fight viral infections.

Keywords: biophysics; self-assembly; computer simulations.

P-225

Preliminary results on human T-cell lymphotropic virus HTLV 1/2 identification among the blood donors

Spinu Igor*[1], Guriev Vladimir[1], Spinu Constantin[1]

(1) Viral Disease Control Center. National Center for Public Health. Chisinau, Republic of Moldova.

Currently, habilitate requirements submitted by the structures of the EC to the service of blood include testing of mandatory donated blood, blood components, including stem cells to the presence of markers of viral hemotransmisibile infections: infection with viral hepatitis B, C, HIV infection, cytomegalovirus and infection with HTLV-1 and HTLV-2 (Human T-cell lymphotropic virus HTLV ½).

Laboratory investigations algorithm in national blood service achieved by the insurance for the biosafety risk exclusion from the hemotransmisibile transmitting of viral etiology infections includes testing of the donors for human imunodificiency virus and viral hepatitis B and C. In this context a special scientific-practically interest presents data to presence of this Human T-cell lymphotropic virus among on blood donors. The results show that during investigation of blood samples collected from donors in number of 258 people aged 19 to 59 years, five persons were HTLV positive. Preliminary obtained results allowing us to following:

- to organize together with donor service additional researches on markers HTLV for donors of blood.

- studying the possibility of supplementing the donors blood investigation on infections by viruses hepatitis B, C and HIV, with additional HTLV virus, in the National Centers for blood transfusion, reducing the risk of post-transfusional disease, caused by the named virus.

- extending these studies and the other contingents of population in particular with increased risks of infection with hepatitis viruses B, C and HIV, taking into account the fact that associate ways of transmission.

Keywords: HTLV-1/2, hemotransmisibile infections, blood donors.

P-226

Role of HIV-1 VPU viroporin in potassium transport through plasma membrane

María Eugenia González Portal*[1], Laura Herrero[1]

(1) Centro Nacional de Microbiología. Instituto de Salud Carlos III. Madrid.

Human immunodeficiency virus type 1 (HIV-1) Vpu is an accessory protein that belongs to the viroporin family. Viroporins interact with cell membranes enhancing virus growth and also membrane permeability. Pore-like and ion channel structures have

been proposed for the retroviral viroporin. Accordingly, *in vivo* studies have shown that Vpu protein increases the membrane permeabilization. Electrophysiological methods have revealed an ion channel activity for Vpu protein; besides, computer simulations pointed to weak cation selectivity of the Vpu channel. Nonetheless, the molecular mechanism of Vpu at the plasma membrane is still uncertain.

The yeast *Saccharomyces cerevisiae* is a useful genetic model system to analyze the functioning of heterologous ion channels. This eukaryotic model allows expression of integral membrane proteins at high levels in an inducible manner. Hence, we used a *wild-type* strain and the isogenic yeast strain carrying *trk1, trk2* mutations to characterize how Vpu protein impacts cellular ion homeostasis. Vpu expression impairs cell growth in the *wild-type* strain but fully rescues the defective growth phenotype of the yeast potassium uptake mutant. Viral protein activity is dependent on extracellular K^+ concentration and pH. Additionally, the growth restitution in mutant strain was more evident in the absence of extracellular ammonium. These data show that Vpu modifies cellular growth of both yeast strains by interfering with the K^+ transport through plasma membrane. The sensitivity to the aminoglycoside antibiotic has been proposed as an indirect indicator of the yeast plasma membrane potential. We noted that Vpu confers tolerance to hygromycin B in *trk1,2* mutant. This reduction in hypersensitivity is consistent with our expectation of a Vpu-mediated depolarization of the plasma membrane in the K^+ uptake mutant. The increased inward transport of cations by Vpu might rescue the resting membrane potential in K^+ uptake-deficient cells. In contrast, the depolarizing effect of Vpu on *wild type* cells might impair ion homeostasis. Our results thus suggest that Vpu may function as a low affinity cation channel modifying inward currents of K^+ and also NH_4^+.

Keywords: HIV-1, viroporins, membrane permeability

P-227

Ratjadone A inhibits HIV by blocking the CRM1-mediated nuclear export pathway

Eric Fleta Soriano*[(1)], Javier P. Martinez[(1)], Ronald Frank[(2)], Florenz Sasse[(2)], Andreas Meyerhans[(1)]

(1) CEXS. UPF. Barcelona (2) Department of Chemical Biology. Helmholtz Centre for Infection Research . Braunschweig, Germany.

Human Immunodeficiency Virus (HIV) infections remain a major health threat worldwide. Although current antiretroviral treatment (ART) is effective in controlling the infection, it is not able to cure HIV due to the development of drug-resistance and latent reservoirs. Targeting host-factors involved in HIV replication is an appealing alternative that might help to overcome the problems with current ART. Here we tested Ratjadone A, an inhibitor of the cellular protein CRM1 (Exportin 1). CRM1 is needed for the Rev-mediated export of

HIV transcripts into the host-cell cytoplasm. Ratjadone A inhibits HIV *in vitro* with an effective concentration 50 (EC_{50}) of 1.52nM. The inhibitory effect of Ratjadone occurs around 12 hours post-infection, coinciding with the HIV-RNA nuclear export step. It was confirmed by a drug affinity responsive target stability (DARTS) assay that Ratjadone A binds to CRM1 but not HIV-Rev. Finally, it is shown here that the drug inhibits specifically the CRM1-mediated HIV-RNA nuclear export pathway. These results demonstrate that Ratjadone A efficiently inhibits HIV infection *in vitro* and that it is conceptually possible to inhibit HIV by targeting host proteins.

Keywords: ratjadone A; HIV; CRM1; host factor.

P-228

Phylogeographic analyses on the HIV-1 subtype G Iberian variant support its ancestry in Cameroon and its propagation from Portugal to Spain through multiple introductions

Aurora Fernández García[1], Elena Delgado[1], Yolanda Vega[1], Ricardo Fernández Rodríguez[2], Carlos Gustavo Cilla[3], Antonio Ocampo[5], Ana Mariño[4], Vanessa Montero[1], Lucía Pérez Álvarez[1], Miguel Thomson*[1]

[1]Centro Nacional de Microbiología. Instituto de Salud Carlos III Majadahonda, Madrid [2] Servicio de Medicina Interna. Complexo Hospitalario Universitario de Ourense. [3]. Servicio de Microbiología Hospital Donostia. [4]. Servicio de Medicina Interna. Hospital Arquitecto Marcide. Ferrol, La Coruña. [5] Servicio de Medicina Interna Complexo Hospitalario Universitario de Vigo.

In contrast to other Western European countries, where subtype B is greatly predominant among HIV-1 strains transmitted in the local population, in Portugal approximately 30% HIV-1 infections are caused by a monophyletic subtype G variant, which is circulating locally among the native population. This variant also circulates at lower prevalences in Spain, mainly in the Northwestern region of Galicia, with sporadic cases in other Western European countries. Here we analyze, by using a Bayesian method, the origin of this variant (Iberian G or G_{IB}) and its temporal-spatial propagation dynamics. Previously, through BLAST searches using protease-reverse transcriptase sequences and phylogenetic maximum likelihood analyses, we identified 4 Cameroonian viruses closely related to G_{IB}. These were included in the alignment used for the Bayesian analysis, together with other subtype G viruses from Africa (n=65, from 8 countries), Portugal (n=25) and Spain (n=23). SRD06 codon position substitution model, lognormal relaxed clock, and Bayesian skyride demographic growth model priors were used in the analysis. Diffusion pathways were analyzed through a Bayesian stochastic search variable selection (BSSVS) approach. The posterior distribution of trees, summarized in a maximum clade credibility tree, showed that viruses from Portugal and Spain formed a monophyletic clade (G_{IB}) supported by a posterior probability (PP) of 1, in which Portuguese and Spanish viruses were interspersed. The 4 above-mentioned Cameroonian viruses grouped in a sister clade of G_{IB}, joining it with PP of 1, with 10 other

Cameroonian viruses being the next African isolates most closely related to G_{IB}. The time of the most recent common ancestor of G_{IB} was estimated in 1987 and its split with its Cameroonian sister clade in 1984. The Cameroonian ancestry of G_{IB} was supported with a posterior state probability (PSP) of 0.82 and its origin in Portugal with a PSP of 0.84. BSSVS analysis supported two separate introductions from Portugal to Spain, with the Galician city of Vigo (near the Portuguese border) being the main center of G_{IB} dispersion in Spain. The Bayesian skyride plot indicated exponential growth in the effective number of infections of the G_{IB} variant from 1995 to 2000, with subsequent stabilization. The results therefore indicate that G_{IB} derives from a Cameroonian strain, and that it originated in Portugal in the mid-1980s, with subsequent diffusion to Spain through multiple introductions.

Keywords: HIV-1, subtype G, Portugal, Spain, phylogeography.

P-229

Role of four mutations in the Human Immunodeficiency Virus envelope gene from the virus of a group of non progressor patients in viral replication

Ana Maceira*[1], Cecilio López Galíndez[1], Concepción Casado Herrero[1]

[1] Departamento de Virología Molecular. Centro Nacional de Microbiología, Majadahonda. Madrid.

Background: Using phylogenetic analysis, the Molecular Virology Laboratory of the "Centro Nacional de Microbiología" (ISCIII) identified a group of six individuals infected in the 80´s with the same ancestral virus variant of human immunodeficiency virus (HIV). All individuals included in the group, had common epidemiological characteristics as they share the same geographical location (Madrid, Spain), chronological time (end of 80´s) and transmission route (intravenous drug practices (IDU).

Clinical monitoring and follow up was possible in five of these patients and has allowed the classification of patients as Long Term Non-progressor patients (LTNPs). Four of them had viral load below 50 copies/ml, and then should be considered as Elite controllers. Cloning the envelopes genes from four of the patients produced recombinants viruses unable to replicate except from one of the patient (designated AS7). The envelope of this virus showed 4 mutations in comparison with the non-replicating viruses. These mutations mapped in the gp120 protein and according to the position of nucleotide sequences in the envelope gene were T419C, T836C, T1199C, and G1502A.

Objectives: To analyze the involvement of these mutations in the fitness recovery observed in the AS7 virus.

Materials and Methods**:** The mutations described in patient AS7, were introduced by PCR mutagenesis in an AS7 clone, first individually (single mutants) and then in combination of mutations for double, triple and quadruple mutants. Recombi-

nant viruses with the mutations were obtained by transfection in 293T cells and then used to biological characterization of the mutants by infection of U87-CCR5 and TZM cells. Virus production monitoring was carried out by p24 antigen, reverse-transcriptase enzyme-activity and viral titration.

Results: The single mutants T836C and G1502A showed a decrease in replication capacity in comparison with the original AS7 virus. Therefore, the four mutations studied appear to be involved in the fitness recovery detected in virus As7 when compared with the other cluster viruses.

Keywords: HIV, mutations, low replication, LTNP, pathogenicity.

P-230

Synergistic activity of carbosilane dendrimers in combination with antiretroviral drugs against HIV

Enrique Vacas Córdoba[1], Marjorie Pion[1], Eduardo Arnáiz[2], Francisco J. de la Mata[2], Rafael Gómez[2], María Ángeles Muñoz Fernández[1]

(1) Laboratorio Inmunobiología Molecular. Hospital General Universitario Gregorio Marañón. Madrid (2) Inorganic Chemistry Department. UAH . Alcalá de Henares, Madrid.

Self-administered topical microbicides or oral pre-exposure prophylaxis could be very helpful tools for women and homosexual collectives to decrease the HIV-infection rates. Anionic carbosilane dendrimers have been shown as safety and effective compounds against HIV with great potential as topical microbicides. However, nowadays, antiretroviral (ARV) drugs are the most advanced microbicides. Due to most of the attempts done to develop effective topical microbicides have failed, combinatorial strategies could be valid approaches in the design of new microbicides.

We evaluated various combinations of anionic carbosilane dendrimers G3-S16 and G2-NF16 with different ARV against CXCR4 (-X4) and CCR5 (-R5) viral strains in TZM.bl human cell line and PBMC. G3-S16 and G2-NF16 dendrimers showed a synergistic activity profile with zidovuline, efavirenz, maraviroc and tenofovir in the majority of combinations tested against X4 and R5 tropic HIV-1 in cell lines as well as in primary human cells.

Therefore, we demonstrated that the combination of ARV with anionic dendrimers enhances the antiviral potency of the individual compounds and supports further clinical research on these combinations as potential microbicides in the context of blocking HIV-1 sexual transmission.

Keywords: HIV, dendrimer, ARV, microbicide, synergy.

P-231

CoRISpe, the Spanish cohort of HIV infected children: current situation

Maria Luisa Navarro Gómez*[1], María Isabel González-Tomé[2], Santiago Jiménez de Ory[3], Pere Soler Palacín[4], María Espiau Guarner[4], Grupo de Trabajo CoRISpe[5]

[1] Sección de Enfermedades Infecciosas. Servicio de Pediatría. Hospital General Universitario Gregorio Marañón. Madrid [2] Unidad de Inmunodeficiencias Pediátricas, Servicio de Pediatría. Hospital 12 de Octubre. Madrid [3] Laboratorio de Inmunobiología Molecular. Hospital General Universitario Gregorio Marañón. Madrid [4] Unitat de Patologia Infecciosa i Immunodeficiències de Pediatria. Hospital Universitari Vall d` Hebron. Barcelona [5] CoRISpe. Spain.

Background and aims: CoRISpe, a Spanish cohort including HIV-infected pediatric patients has been recently created, yielding interesting information about the current situation of HIV-infected children in our country, which may be extrapolated to other developed countries.

Methods: A cross-sectional study was performed. Data were collected until December 2011.Most recent CD4 (count or percentage depending on patient´s age) and plasma viral load were evaluated.

Results: The CoRISpe has recruited 838 patients. 302 are over 18 years old. 536 HIV-1-infected children followed in 53 different hospitals were studied. The median age was 12,6 years (range:0,04-17,98). Vertical transmission was the dominant mode of acquisition of infection (97%). 53,7% were female. Most of the children were born from Spanish parents (67,9%) but in the last years an increasing number of patients were immigrants or were born of immigrants,mainly in Subsaharan Africa (19,6%) and South America (7,5%). 4,6% of patients has Hepatitis C Virus (HCV). 23% of the children had developed AIDS. 95% were receiving HAART,and 5% were without treatment. The most common regimen included two nucleoside reverse transcriptase inhibitors (NRTI) plus a protease inhibitor (PI) (46,6%).The most used antiretroviral were Lopinavir/r (51,1%), 3TC (40%), ABV (39,1%) and FTC (30,2%). Median CD was 834/mm3 (IQR: 601-1143) and median CD4% was 33,3% (IQR:28,2-38), most of the children had a CD4% over 25% (88,8%) and CD4 over 500/mm3 (86,7%). 74,1% of the patients receiving HAART had an undetectable plasma viral load.

Conclusions: The institution of a National Cohort has led to an improve in the knowledge of paediatric HIV infection in Spain. In the last years an increasing number were immigrants. A high proportion of vertical-infected children are becoming adolescents and adults. Most of the children were receiving HAART and they had a good immunological response.

Keywords: HIV, pediatric, epidemiology, cohorts.

P-232

Phylogenetic surveillance of HIV-1 genetic diversity in two regions of Spain: Galicia and Basque Country (2001 – 2012)

Elena Delgado*[1], Yolanda Vega[1], Aurora Fernández García[1], Vanessa Montero[1], Ana Sánchez[1], Lucía Pérez Álvarez[1], Michael M Thomson[1], Study Group of HIV-1 Antiretroviral Resistance in Galicia and Basque Country[2,3]

[1] HIV Biology and Variability Unit. Instituto de Salud Carlos III.Majadahonda, Madrid [2] Conselleria de Sanidade. Xunta de Galicia. Santiago de Compostela [3] Osakidetza. Servicio Vasco de Salud. Bilbao.

Background: HIV-1 displays high genetic diversity. Subtype B is the major HIV-1 genetic form in Western European countries. Trend changes in immigration or transmission route could modify the HIV-1 genetic diversity in a geographic area.

Objective: To analyze changes in the distribution of HIV-1 genetic forms in two regions of Spain along twelve years.

Methods: Protease-reverse transcriptase was (RT)-PCR amplified from plasma-derived RNA or whole blood-derived DNA, collected from HIV-1 infected subjects in 2001-2012 in two regions of Spain (3137 from Galicia and 2996 from Basque Country). Phylogenetic analyses were performed via maximum likelihood with RAxML. Recombination was analyzed by bootscanning.

Results: We identified 904 (14.7%) non-subtype B infections among 6133 HIV-1 infected patients: 477 (15.2%) in Galicia and 427 (14.3%) in Basque Country. In patients HIV-1 diagnosed since 2010, the prevalence of non-B genetic forms increased to 32.1% and 27.6% in Galicia and Basque Country, respectively.

Non-subtype B distribution in Galicia is dominated by subtypes F1 (2.93%) and G (1.40%) and their corresponding recombinants with subtype B: BF (1.47%) and BG (3.32%), followed by subtype C (1.75%), CRF02_AG (1.63%) and A (0.73%). In the Basque Country, CRF02_AG is the most prevalent non-B genetic form (5.37%), followed by subtypes C (1.20%), F (0.93%), G (0.93%) and A (0.77%).

In Galicia, 70% and 3% of CRF14_BG infections were diagnosed before 2004 or after 2010, respectively, while subtype F infections increased from 3% to 84% between both periods. Transmission route trends are associated with these changes. CRF14_BG propagated mainly among injected drug users, while subtype F is expanding in a large transmission cluster among men having sex with men (MSM), which is the major transmission route since 2005.

Geographic origin of the patients infected with non-B genetic forms is unequally distributed in both regions: In Galicia, 56% of non B infections were in Spaniards versus 34% in Basque Country, while 31% corresponded to immigrants in Galicia, versus 64% in Basque Country.

Conclusion: Notable regional and temporal variations in the distribution of HIV-1 genetic forms in two regions of Spain are reported. Although the overall prevalence of HIV-1 infections with non-B

genetic forms in 2001-2012 was 14.7%, currently it has increased to 30%, with a remarkable expansion of a subtype F cluster among MSM in Galicia.

Keywords: HIV, subtypes, genetic diversity, phylogenetyc surveillance.

P-233

Validation of a procedure for virus detection in green onions

Noemi Fuster*[1], Rosa M Pintó[1], Albert Bosch[1]

[1] Department of Microbiology. University of Barcelona.

The viruses primarily associated with foodborne illness are norovirus (NoV), causing gastroenteritis, and hepatitis A virus (HAV), the etiological agent of the most common type of hepatitis. Fresh produce, including green onions, is implicated more and more in diffuse outbreaks of foodborne illness because it usually undergoes little, if any, processing before consumption. The largest documented hepatitis A outbreak in US history was linked to consumption of green onions harvested in Mexico and caused three casualties.

Our lab has decisively contributed within the framework of a technical advisory group of the European Committee on Standardization (CEN) to the development of a standard method for detection of norovirus (NoV) and hepatitis A virus (HAV) in selected foodstuffs. The CEN committee developed ISO proposals for sensitive and quantitative RT-PCR based methods for the detection of HAV and NoV that could enable the formulation of regulatory standards for viruses in food.

In the present report, data from validation of the procedures on spiked and naturally contaminated green onion samples are presented. Initially, uncontaminated (tested negative for viruses) 25-g samples were spiked with three different levels of genome copies ($5.0x10^5$, $5.0x10^6$ and $5.0x10^7$) of NoV GI and GII, and HAV. The efficiency of virus recovery was assessed to range from 28 to 48%.

The same standardized procedure was employed for the determination of viruses in naturally contaminated green onion samples from different points in the Daqahlia governorate, around the city of El Mansura in the Nile delta, Egypt. Viruses were detected in 34% (49/144), with mean virus numbers of $5.6x10^2$ genome copies per gram.

Available reliable standardized assays for virus detection in food matrices including appropriate quality assurance and quality control measures to assess the efficiency of critical steps in virus analysis open the possibility to produce consistent and accurate exposure data to be used in QMRA and at the same time may enable the formulation of guidelines to ensure the virological quality of selected commodities in specific scenarios to reduce the risk of foodborne virus infections.

Keywords: foodborne virus assay, norovirus, hepatitis A virus, mengovirus.

P-234

A novel class of DNA satellites associated with New World begomoviruses infecting malvaceous plants

Elvira Fiallo Olivé*[1], Yamila Martínez[2], Enrique Moriones[1], Jesús Navas Castillo[1]

(1) Departamento de Interacción Planta Patógeno. Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" (IHSM-UMA-CSIC). Málaga (2) Departamento de Fitopatología. Centro Nacional de Sanidad Agropecuaria. Mayabeque, Cuba.

Begomoviruses (genus *Begomovirus*, family *Geminiviridae*) are whitefly (*Bemisia tabaci*, Hemiptera: *Aleyrodidae*)-transmitted plant ssDNA viruses that cause serious diseases in a number of economically important crops, mostly in tropical and subtropical regions. They have been shown to be helper viruses for a number of distinct DNA satellites, including betasatellites and alphasatellites. Replication of the satellites interferes to some degree with replication of the helper and in some cases they affect the disease symptoms. To date, betasatellites and related molecules such as the satellite associated with *Tomato leaf curl virus* (ToLCV-sat), have only been associated with Old World begomoviruses. During a survey in Cuba, we found *Malvastrum coromandelianum* and *Sidastrum micranthum* (family *Malvaceae*) plants infected with bipartite begomoviruses associated with ssDNA molecules of a quarter the size of the viral genome components. The molecular characterization of these molecules showed that, in addition to sharing some genetic features with betasatellites and ToLCV-sat such as an A-rich region, contained nucleotide stretches of begomoviral origin, presumably the remains of recombination events involved in their origin. These molecules are putative DNA satellites which would constitute a novel class of such subviral agents.

Keywords: geminiviridae, begomoviruses, DNA satellites, *Malvaceae*.

P-235

Survey for viruses infecting vegetable crops in Azerbaijan: mosaic, stunting, yellowing, shortening of the internodes

Nargiz Fakhraddin Sultanova*[1], Alamdar Charkaz Mammadov[1], Irada Mammad Huseynova[1]

(1) Department of Fundamental Problems of Biological Productivity. Institute of Botany. Baku, Azerbaijan.

Biotic stresses including viruses may cause many important plant diseases and responsible for considerable losses in crop production and qualityin the world. The remarkable floral biodiversity of Azerbaijan has triggered some recent interest in the biodiversity of endemic plant viruses. In this

purpose field surveys were conducted in the major production areas of Azerbaijan during 2009-2012 growing seasons to identify viruses showing virus like symptoms (mosaic, stunting, yellowing, phloem discoloration and others) in vegetable crops. The study provide proof for the efficiency of combining serological diagnosis (ELISA method) with the power of PCR and DNA rolling circle amplification (RCA) to rapidly obtain genetic information on viral plant pathogens with a circular DNA genome. We carried out investigations on different vegetable samples and determined the following viruses - *Bean leaf roll virus* (Luteovirus, BLRV), *Bean common mosaic virus* and *Bean yellow mosaic virus* (Potyviruses, BCMV, BYMV), *Alfa-alfa mosaic virus* (Bromovirus, AMV), *Tomato mosaic virus*(Tobamovirus, TMV), *Tomato chlorosis virus* (Crinivirus ToCV), *Melon necrotic spot virus* (Carmovirus, MNSV), *Cucumber mosaic virus* (Cucumovirus, CMV) and nanoviruses. DNA of two chickpea (*Cicer arietinum*) and two lentil (*Lens culinaris*) samples with relevant positive results for nanoviruses was amplified by RCA, restricted by endonucleases *Aat*II or *Hind*III and analyzed in 1.5% agarose gel. Restriction by *Aat*II produced predominant 1-kb fragment. This fragment was a strong indication of the presence of a nanovirus infection in these samples. The template DNA of chickpea and lentil samples were also amplified by using specific primer pairs F103/R101 and C5F/C5R for nanoviruses. These samples yielded PCR products of the expected sizes 770 bp and 660 bp, respectively, confirming the presence of nanoviruses. We describe an initial assessment of plant viruses with a single-stranded DNA genome in Azerbaijan. Survey of cultivated and wild crops has uncovered the presence of three different nanoviruses, two distinct *Faba bean necrotic yellows viruses* (FBNYV) and one *Faba bean necrotic stunt virus* (FBNSV). In addition, tomato plants showing typical symptoms of tomato yellow leaf curl virus, a geminivirus, were observed. The following researches will allow finding out new viruses in these crops.

Keywords: plant viruses, vegetables, ELISA, RCA, PCR, single-stranded (ss) DNA viruses.

P-236

Analysis of serological and molecular variability of faba bean necrotic yellows virus isolates from Spain

Elena Navarro[1], Vilma Ortiz[1], Gerardo Carazo[1], Javier Romero*[1]

[1] Departamento de Protección Vegetal. INIA. Madrid.

In all, 33 of 210 samples (15.71 %) collected in 2002-2004 from broad bean plants showing yellowing and leaf deformation in Spain gave nanovirus- positive reactions when analyzed by triple-sandwich enzyme-linked inmunosorbent assay using broad-spectrum monoclonal antibodies (MAbs) specific to nanoviruses. Further analysis of these samples with five discriminating MAbs

revealed divergent epitope profiles that were categorized into three serogroups designed A, B and C. Serogroup A was found in 29 (88 %) isolates, whereas serogroups B and C had only two (6.0 %) isolates each. To obtain a better understanding of this nanovirus variability in Spain, the entire genomes of three isolates: Mu-15C, Mu-14B and Mu-11D representing to serogroups A, B and C, less the DNA-U4 component of MU-14B isolate were sequenced. The eight circular ssDNA components obtained for each isolate, range in size from 986 to 1005 nt for Mu-15C, from 987 to 1005 for Mu-14B and from 984 to 1005 for Mu-11D and were structurally similar to previously described *Faba bean necrotic yellows virus* (FBNYV) DNAs. However, the Spanish isolates differed from each other in overall nucleotide and amino acid sequences. Mu-11D was most closely related to FBNYV isolates described early from Egypt and Syria with which it shared a mean amino acid sequence identity of about 97 %. MU-15C and Mu-14B shared a mean amino acid sequence identity of approximately 98 % but MU-15C only shared a mean amino acid sequence identity of 91 % with FBNYV isolates from Egypt and Syria and Mu-14B of 94 %. On the other hand, the isolate Mu-14B which reacted strongly with the MAbs specific to *Faba bean necrotic stunt virus* (FBNSV), shared a mean amino acid sequence identity of about 78 % with the FBNSV isolates described from Ethiopia and Morocco. Phylogenetic analysis of all eight deduced protein amino acid sequences of Spanish isolates together with those of the other nanoviruses demonstrated that Mu-15C and Mu-14B cluster with FBNYV isolates, while Mu-11D formed a tight cluster with FBNYV Egypt and Syria isolates. Following the guidelines on nanovirus species demarcation, this data suggested that Mu-15C, Mu-14B and Mu-11D represent distinct strains of FBNYV.

Keywords: FBNYV, serology, sequences, phylogenetic.

P-237

RNA silencing suppressors mediate in the stability of their cognate viral coat proteins

Araiz Gallo*[1], Jon Ochoa[1], María Calvo[1], Bernardo Rodomilans[1], José J. Pérez[1], Juan A. García[1], Adrián Valli[1]

(1) Departamento de Genética Molecular de Plantas. Centro Nacional de Biotecnología-CSIC. Madrid.

Plant viruses belonging to different genera of the family *Potyviridae* use diverse RNA silencing suppressors (RSSs) to counteract antiviral RNA silencing responses. Although members of the genus *Potyvirus* depend on the RSS HCPro, it can be functionally replaced in the potyvirus *Plum pox virus* (PPV) by heterologous silencing suppressors as the protein P1b of the ipomovirus *Cucumber vein yellowing virus* (CVYV). HCPro stabilizes its cognate viral capsid protein (CP) and enhances the accumulation of full-size virions, however these abilities do not depend on its RNA silencing suppression activity, and cannot be supplied by other viral RSSs. Although HCPro has a stabilizing effect on CP expressed alone, it is much less pronounced

than the effect on CP expressed as part of a polyprotein, together with other viral proteins. In spite of its lack of similarity with PPV-HCPro, CVYV-P1b is also able to specifically enhance the stability of CVYV-CP in agroinfiltration assays, although at much lower extent than in the viral infection. Using an optimized bimolecular fluorescence complementation system, we were able to detect physical interaction between P1b and CP proteins of CVYV. This interaction is observed in the cytoplasm and in the nucleus. These results reinforce the theory that the stabilization of CP by its cognate RSS, probably by facilitating virion assembly, is a general property of the *Potyviridae* family.

Keywords: plum pox virus, cucumber vein yellowing virus, RNA silencing supressor, CP stability.

P-238

Estimation of multiplicity of cellular infection in mixed infections of Tomato bushy stunt virus and associated defective interfering RNAs

Livia Donaire*[1], Fernando García Arenal[1]

(1) Centro de Biotecnología y Genómica de Plantas UPM-INIA and E.T.S.I. Agrónomos. Universidad Politécnica de Madrid.

The number of viral genomes infecting a cell (or multiplicity of cellular infection, MOI) determines the extent of such phenomena as recombination between viral strains, competition and complementation of defective mutants. These interactions are major players in the evolution and epidemiology of viruses. Despite its importance, estimations of MOI during plant virus infections are limited to three examples, a DNA virus (*Califlower mosaic virus*, CaMV) and two RNA viruses (*Tobacco mosaic virus*, TMV and *Soil-borne wheat mosaic virus*, SBWMV). MOI values in these three studies varied widely, ranging from 1 to 13 over the course of the viral infection in the host plant. Small values of MOI indicate mechanisms preventing co-infection of cells.

Tomato bushy stunt virus (TBSV) is the type member of the genus *Tombusvirus*. The genome of TBSV is a positive sense ssRNA of approximately 4.8 kb which encodes five open reading frames: two proteins involved in replication, the coat protein, the movement protein and a suppressor of gene silencing. Under high multiplicity serial passages, the infection of TBSV (and other *Tombusvirus*) is associated with the appearing of defective interfering RNAs (DI-RNAs). DI-RNAs are incomplete RNA viral genomes which require the present of their helper virus for replication and packaging. The presence of DI-RNAs results in a decreasing of virus titer and usually in an attenuation of the symptoms caused by the helper virus. The high titer of DI-RNAs suggests that mechanisms excluding co-infection with the helper virus could not apply.

In this work we have estimated MOI during the infection of TBSV in the systemic host *Nicotiana*

benthamiana, with or without the presence of DI-RNA molecules in the initial inoculum. Our purpose was to evaluate the possible variation in MOI levels due to the interaction of the helper virus and derived DI-RNAs, coexisting in the same plant cell. For this purpose, we have adopted two technical approaches. On the one hand, we have used two fluorescent tagged viruses to quantify the number of single and double infected cells, which allows the estimation of genotype frequencies and MOI during the viral infection. On the other hand, we have developed a strategy based on single-cell quantitative PCR following by high-resolution melting (HRM) to quantify single and mixed infected cells with TBSV and the associated DI-RNA, DI-72, both labeled with two different genetic markers introduced by molecular engineering.

Keywords: plant viruses, MOI, virus evolution, DI-RNAs.

P-239

No evidence for replication of the begomovirus tomato yellow leaf curl virus in its vector, the whitefly *Bemisia tabaci*

Sonia Sánchez Campos[1], Edgar Rodríguez Negrete[2], Ana Grande Pérez[2], Eduardo R Bejarano[2], Jesús Navas Castillo[1], Enrique Moriones*[1]

[1] Departamento de Protección Vegetal. IHSM-UMA-CSIC. Málaga [2] Departamento de Biología Celular, Genética y Fisiología. IHSM-UMA-CSIC. Málaga.

Geminiviruses (family *Geminiviridae*) are plant viruses with small circular single-stranded DNA genomes that are encapsidated in twinned particles. This family is divided into four genera according to their host-range, genome structure and insect vector. With more than 200 species, the genus *Begomovirus* is the largest genus in this family and comprises viruses infecting dicotyledonous plants that are transmitted by the whitefly (*Hemiptera: Aleyrodidae*) *Bemisia tabaci*. Begomoviruses are an emerging threat worldwide in temperate and tropical regions, causing severe damage to economically important crops such as cassava, cotton or tomato. One of the most devastating diseases affecting tomato crops is tomato yellow leaf curl disease (TYLCD). It is caused by a complex of more than ten begomoviruses species, being *Tomato yellow leaf curl virus* (TYLCV) the most widespread worldwide.

TYLCV is transmitted by *B. tabaci* in a persistent manner and a controversy exists about the propagation of the virus in the insect vector. Although there are some data supporting the existence of transcription of the viral genes, no information is available about the ability of TYLCV to replicate within *B. tabaci*. In the plant cell, TYLCV replication occurs in the nuclei of infected cells via double-stranded intermediates composed by virion-sense (VS) and complementary-sense (CS) strands. Thus, CS strands are present only when the genomic DNA is replicating. To find out whether TYLCV replicates within whiteflies a two-step qPCR method for the specific amplification of

VS or CS strands was assayed in viruliferous-whitefly DNA extracts. The results show that CS strands could be detected in whiteflies but only at levels reflecting acquisition from the viral diet. Evolution of CS and VS levels and CS/VS ratios during time course analyses of viruliferous whiteflies do not support TYLCV replication in the insect.

Keywords: begomovirus, geminiviridae, tomato yellow leaf curl virus, *Bemisia tabaci*, virus replication.

P-240

Targeting heterologous proteins to different cell compartments with a potyviral vector

Eszter Majer*[1], José Antonio Daròs[1]

(1) Instituto de Biología Molecular y Celular de Plantas. CSIC-Universidad Politécnica de Valencia.

Plants produce a wide range of secondary metabolites which are used to protect them against herbivores and microbial pathogens and many of these natural products have medical, pharmaceutical and industrial interest. The production of these valuable natural metabolites sometimes is very costly and biotechnological alternatives are needed. Our viral vector based on Tobacco etch virus (TEV, family Potyviridae) is able to express several proteins in equimolar amounts in tobacco plants. The vector contains a cassette to co-express various heterologous proteins replacing the gene of the viral RNA-dependent RNA polymerase (NIb) which is supplied in trans by the host plant. To show the potential use of this vector in plant metabolic engineering, we co-expressed two interacting transcription factors, Delila and Rosea1 from snapdragon (Antirrhinum majus L.), which in tobacco leaves induced a notable accumulation of anthocyanins, a class of pigments produced by higher plants, which have been associated with protection against a broad range of human diseases. To discern the possibilities of this viral vector in plant metabolic engineering, it is essential to know whether heterologous proteins can be targeted to distinct cellular organelles. In our work, we analyzed the ability of the vector to deliver the green fluorescent protein (GFP) to different cell compartments, like the chloroplast, where a plenty of plant metabolic pathways occur, or the endoplasmic reticulum, an important step of secretory pathway. We constructed recombinant viral clones in which GFP with or without the transit peptide of Rubisco small subunit from tobacco, and with or without the signal peptide of pectate lyase from tobacco, were expressed either in the amino-terminal end of the viral polyprotein or inside the viral polyprotein replacing the NIb cistron. Our results indicate that the GFP containing the chloroplastic transit peptide enters this organelle only when it is expressed as the amino-terminal product of the viral polyprotein. This result demonstrates a new site to express heterologous proteins from potyviral vectors and that subcellular sorting is possible.

Keywords: plant virus, viral vector, metabolic engineering.

P-241

The VPg of Plum pox virus strain C (PPV-C) is a major pathogenicity determinant that prevents the infection of resistant herbaceous hosts

María Calvo*[1], Juan Antonio García[1]

(1) Departamento de Genética Molecular de Plantas. Centro Nacional de Biotecnología-CSIC. Cantoblanco, Madrid.

PPV-SwCM is an isolate of the uncommon PPV-C strain that in contrast to isolates of other PPV strains, such as PPV-R, is not able to infect important experimental hosts such as *Arabidopsis thaliana* or *Chenopodium foetidum*. The nature of this resistance, which impedes viral replication on inoculated leaves and therefore prevents the onset of a systemic infection, is still unknown. It is also unknown which viral protein(s) trigger(s) or is(are) involved in this mechanism.

In order to answer this last question, we have constructed chimeras between PPV-SwCM and PPV-R by single or multiple exchanges of the coding sequences of the different viral proteins.

Our preliminary results indicate that only the substitution of the VPg cistron of PPV-R by the corresponding sequence of PPV-SwCM impedes the infection of PPV-R both in *A. thaliana* and *C. foetidum*. However, to gain infectivity, PPV-SwCM does not only need the VPg coding sequence of PPV-R, but also additional viral sequences, which are different in the case of *C. foetidum* and in *A. thaliana*.

The fact that PPV-SwCM itself is able to establish a strong systemic infection in other herbaceous hosts, such as *Nicotiana* species, suggests that the replication of the virus relies on an interaction between a host-specific factor and VPg, in which other strain-specific viral proteins are also involved.

Keywords: plum pox virus, pathogenicity determinants, VPg.

P-242

Mechanistic divergence between P1 proteases of the family *Potyviridae*

Bernardo Rodamilans*[1], Adrián Valli[2], Juan Antonio García[1]

(1) Departamento de Genética molecular de plantas. Centro Nacional de Biotecnología – CSIC. Madrid
(2) Department of Plant Sciences. University of Cambridge, UK.

P1a and P1b are two serine proteases of the ipomovirus *Cucumber vein yellowing virus* (CVYV). They belong to the group of P1 factors present at the N-terminus of the polyproteins of most members of the family *Potyviridae*. Besides its proteolytic activity, P1b acts a silencing suppressor in

CVYV. Other functions of P1a remain unknown. Both proteins bear the catalytic triad formed by histidine, aspartic and serine that is characteristic of the serine protease family. There is no structural or mechanistic information available about P1a or P1b, and the functional importance behind their sequence similarity to other potyvirid P1 proteins remains unclear. We have compared the protease activities of P1a and P1b in different experimental systems. The findings made regarding how these two proteases work, such as the requirement of a host factor by P1a but not by P1b, underscore important differences in their catalytic activity that point towards their undergoing divergent evolution involving the acquisition of mechanistic variations. The expression of several truncated forms of P1b *in bacteria* and *in planta* helped define the protease domain of P1b, along with other important features such as its apparently *in cis* mode of action. Recent phylogenetic data, together with the present results, allow an appealing hypothesis to be proposed regarding P1 evolution and its involvement in potyvirid speciation.

Keywords: P1 proteases, potyviridae.

P-243

Effect on the development of *Arabidopsis thaliana* by Turnip mosaic virus

Silvia López González*[1], Flora Sánchez[1], Pilar Manrique[1], Pablo González[1], John Walsh[2], Carol Jenner[2], Pablo Lunello[1], Fernando Martínez[1], XiaoWu Wang[1], Fernando Ponz[1]

(1) Departamento de Biotecnología de Virus. Centro de Biotecnología y Genómica de Plantas UPM-INIA. Pozuelo de Alarcón, Madrid (2) Warwick HRI, University of Warwick, UK.

Turnip mosaic virus (TuMV) belongs to the genus *Potyvirus* in the family *Potyviridae*. The TuMV genome is composed of a positive-sense single-stranded RNA molecule of about 10 kb in length. TuMV is a potyvirus with a very wide host range (that includes over 300 plant species) including the plant model system *Arabidopsis thaliana* and another cultivated plants of great economical interest.

TuMV infections have very distinct symptoms, like the classical necrosis, mosaic, chlorotic lesions, yellowing of leaves and vein clearing, as well as development and growth alterations. The isolates from genetic strain MB (UK1) strongly arrest growth and development, while the isolates MR (JPN1) show a delay in growth and an alteration in the development of the flower stem and the global architecture of the plant, which implies a strong loss of apical dominance and an abnormal pattern in the flower emergence kinetics.

The differential symptoms, especially those that affect the pattern of development in the plant, have been analyzed in a comparative manner in the infections produced by both, the UK1 and the JPN1 isolates. Furthermore, these symptoms are associated to tissue and cellular alterations that differ greatly in both infections. Another important aspect in symptoms differentiation is the identification of the main determinant of symptoms, would provide us a better understanding of the interaction between these biological systems. As in previous research in virus/plant interaction fields, the patosystem TuMV/Arabidopsis can be a very useful instrument for the study of specific aspects in plant development.

Keywords: TuMV, *Arabidopsis thaliana*, infection, development alteration.

P-244

Citrus tristeza virus p23, a multifunctional protein with preferential nucleolar localization: identification of two potential host interactors in a yeast two hybrid screening

Susana Ruiz-Ruiz*[1], Salvatore Walter Davino[1,2], Silvia Ambrós[3], Luis Navarro[3], Pedro Moreno[3], Leandro Peña[3], Ricardo Flores[1]

(1) Department of Plant Stress Biology. Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC), Universidad Politécnica de Valencia. (2) Plant Pathology and Microbiology Division. University of Palermo. Italy (3) Departamento de Protección Vegetal y Biotecnología. Instituto Valenciano de Investigaciones Agrarias. Moncada, Valencia.

The genome of *Citrus tristeza virus* (CTV), of the genus *Closterovirus* within the family *Closteroviridae,* consists in a (ss)RNA (+) of about 19.3 kb (the largest reported for a monopartite plant virus) that it is organized in 12 open reading frames (ORFs) encoding potentially at least 17 proteins. Unique among them is p23, encoded by the 3'-terminal ORF, because it has no homologues in other closteroviruses including the type species of the genus *Beet yellows virus*. CTV-p23 is an RNA-binding protein of 209 amino acids with a putative Zn-finger domain and some basic motifs that accumulates in nucleolus and Cajal bodies (as well as in plasmodesmata), being in this respect the first closterovirus protein with a nucleolar localization signal (recently dissected and shown to be bipartite). Moreover, p23 mediates many functional roles that comprise the asymmetrical accumulation of CTV RNA strands, intracellular suppression of RNA silencing, induction of certain CTV-specific syndromes, and elicitation of CTV-like symptoms and enhancement of systemic infection (and virus accumulation and release from the phloem) when expressed ectopically as a transgene in several *Citrus* spp. These very diverse features make p23 a very attractive candidate for studying its interactions with host proteins. As a first approach to this aim, we have search an expression library of *Nicotiana benthamiana* (wherein CTV is able to replicate and incite symptoms), using the

yeast two-hybrid (Y2H) system. The initial Y2H screening resulted in the identification of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and a transducin/WD40 domain protein, whose interactions with p23 were later confirmed in 1-by-1 Y2H assays. Previous data indicate that the RNA-binding function of GAPDH (a metabolic enzyme) is co-opted by a tombusvirus to convert the host cell into a viral factory (Wang and Nagy, *PLoS Pathogens* 2008). On the other hand, WD40 (also called WD-repeat) domains are common in proteins mediating protein–protein interactions like those involved in scaffolding and in the cooperative assembly and regulation of dynamic multisubunit complexes (Stirnimann et al., *Trends Biochem. Sci.* 2010). Using *in planta* approaches, we next want to confirm the interactions of p23 with these two host proteins and elucidate subsequently the role they may have in CTV biology.

Keywords: closterovirus, yeast two-hybrid, glyceraldehyde 3-phosphate dehydrogenase, transducin/WD40 domain protein.

ÍNDICE DE AUTORES / AUTHOR INDEX

ÍNDICE DE PALABRAS CLAVE / KEYWORDS

EMPRESAS COLABORADORAS SEV

ENTIDADES COLABORADORAS XII CNV