Influenza virus non-structural protein NS1 cooperatively binds virus-specific (+)-strand RNA sequences

Daniel Marc, Denis Soubieux

► To cite this version:

Daniel Marc, Denis Soubieux. Influenza virus non-structural protein NS1 cooperatively binds virus-specific (+)-strand RNA sequences. 15. International Conference on Negative Strand Viruses (NSV 2013), Jun 2013, Grenade, Spain. hal-02744878

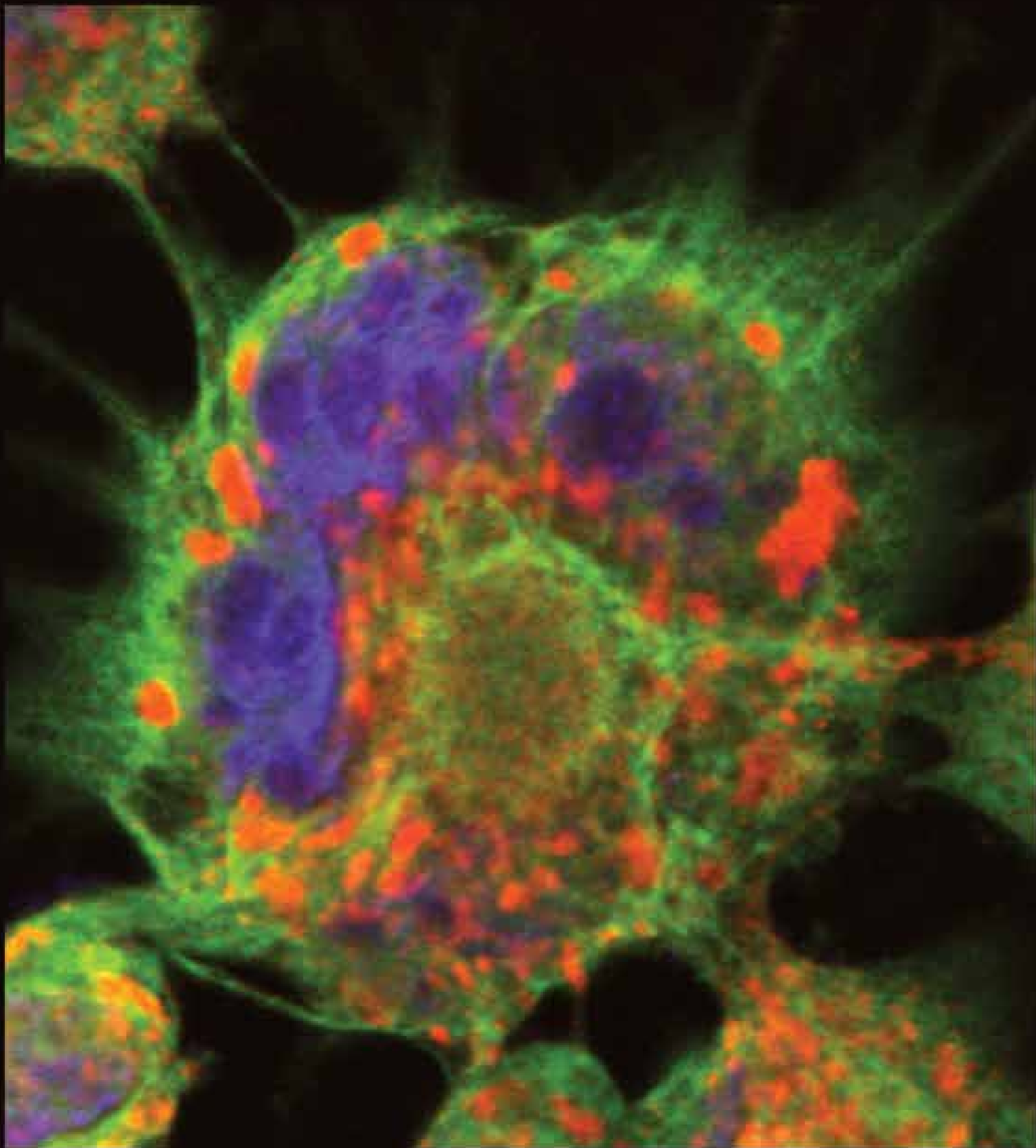
HAL Id: hal-02744878
https://hal.inrae.fr/hal-02744878

Submitted on 3 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

XV International Conference on
Negative Strand Viruses



16 - 21 June 2013, Granada, Spain

The front cover shows a middle optical section of MDCK cells infected with Sendai virus in the process of forming a limited syncytium. The red stain corresponds to the nucleocapsid protein and the greento a M protein mutated so it does not reach the cell plasma membrane. The nuclei are colored with DAPI.

15th International
Negative Strand Virus Meeting

NSV 2013

16 - 21 June 2013, Granada, Spain

Table of Contents

General information 4

Social programme 5

Program sumary 6

Programe 9

Abstracts 45

Authors 223

Registered participants 237

General Information:

Badges
All participants and accompanying persons must wear the oficial badges in order to get Access to the lecture halls and/or social activities.

Official Language
The official language of the Conference is English. Simultaneous translation will not be provided.

Audiovisuals
All presentations are to be uploaded in the conference room. Hand in your PowerPoint presentations to the technicians at least one hour before your talk .

Poster Presentations
Posters in sessions I to V:
These posters are to be mounted between 14.00 hrs and 19.00 hrs on Sunday afternoon, June 16.
These posters are to be removed during the morning coffee break (11:30- 12:30) on Wednesday, June 19.
Posters in Sessions VI to VIII :
These posters are to be mounted during the lunch break (13:50 – 15:20) on Wednesday, June 19.
These posters are to removed between 12:00 and 14:00 on Friday , June 21.

Conference Documents
Participants will receive a badge , a certificate of attendance and a conference bag with conference- related documents at the registration desk.

Meals
Lunches will be provided in the Palacio de Congresos , as indicated in the programme. Evening meals will be provided as part of the social programme. The cost of these are included in the registration fee.

Non-Smoking Policy
In Spain you are only allowed to smoke outside buildings.

Granada's city buses

Granada's urban bus network is managed by the Transportes Rober bus company. Bus network maps, timetables, fares, and also buses to the Alhambra and Albayzin etc are available on

Granada's city buses such.

Granada's taxis

You can find taxis at exit of bus stations, trains and airports. Granada is a small city, and it is easy to get around, taxis are not very expensive.

Tele - Radio - Taxi Granada
Telephone: +34 958 28 06 54

Radio Taxi Company:
Telephone: +34 958 13 23 23

Spanish timing and habits

Breakfast: Between 8am - 10am
Lunch: Kitchen service is usually open in restaurants between 1pm - 4pm, although some offer food even earlier
Dinner: Usually from 8.30pm until 11pm

Climate
The climate in Granada is Continental Mediterranean. Temperatures are fresh in Winter, and **hot in summertime**, reaching maximuns above 35ºC (95ºF). Check the weather in the **Spanish Agency of Meteorology**

Social Programme

Opening Reception on Sunday , June 16
The Opening Reception will take place at Palacio de Congresos at 20.00 hrs.

Address :

Walking Guided Tour of Granada on Monday , June 17. And free evening.
The walking tour will start from the Palacio de Congresos at 19.30 hrs. And will end at approx 21.00 hrs.

Alhambra's visit + dinner in the gardens overlooking Granada by night.
The Alhambra visit start from Palacio de Congresos at 19.30 hrs.

Gala Dinner on Thursday , June 20
The Gala Dinner will take place in "La Mamounia". Buses start from Palacio de Congresos at 20.00 hrs.

Program Sumary:

Sunday June 16

14:00 - 19 :00	Registration Palacio de Congresos, Put up posters for sessions 1-4
19:00-23 :00	Opening reception Palacio de Congresos
20:00	Flamenco concert: roof of Palacio de Congresos

Monday June 17

09:45-10:00:	Welcome and introductory information
	Session I:In and Out and Moving About (10:10- 13:50)
10:00 -10:10	Chairpersons's remarks
11:30 -12:30	Coffee break-Poster session
13:50 -15:20	Lunch- Poster session
	Session II: RNA synthesis and gene expression (15:30-18:50)
15:20-15:30:	Chairpersons's remarks
16:50 -17 :50	Coffee break-Poster session
19:30	Guided tour of Granada and free evening

Tuesday June 18

	Session III:Structure and Function (10:10-13:50)
10:00-10:10:	Chairpersons's remarks
11:30 -12 :30	Coffee break-Poster session
13:50-15:20	Lunch- Poster session
	Session IV:Host-cell interactions (15:30-19:10)
15:20-15:30:	Chairpersons's remarks
16:50 -17 :50	Coffee break-Poster session
20:00	Alhambra's visit + dinner in the gardens overlooking Granada by night

Wednesday June 19

09:00-10:00	Put up posters for sessions V-VIII
	Session V: Antiviral response (10:10-13:50)

10:00-10:10:	Chairpersons's remarks
11:30 -12:30	Coffee break-Poster session
13:50-15:20	Lunch- Poster session
	Session VI: Pathogenesis (15:30-19:00)
15:20-15:30	Chairpersons's remarks
17:00 -18:00	Coffee break-Poster session
	Free evening

Thursday June 20

	Session VII: Vaccines and antivirals (10:10-13:20)
10:00-10:10:	Chairpersons's remarks
11:30 -12 :30	Coffee break-Poster session
13:20-15:00	Lunch- Poster session
	Session VII:Vaccines and antivirals continued (15:10-17:50)
15:00-15:10	Chair persons's remarks
16:10 -17 :10	Coffee break-Poster session
20:00	Gala dinner .

Friday June 21

	Session VIII: Natural and unnatural virus evolution (09:10-12:10)
09:00 - 09:10	Chair persons's remarks
10:00 -11 :00	Coffee break-Poster session
12:10	Lunch and beer session

Program

Sunday June 16

14:00- 19 :00	Registration Palacio de Congresos, Put up posters for sessions 1-4
19:00-23 :00	Opening reception Palacio de Congresos
20:00	Flamenco concert: roof of Palacio de Congresos

Monday June 17
09:45-10:00: Welcome and introductory information
Session I: In and Out and Moving About
Chairpersons :Christina Spirolopulo and Benhur Lee
10:00-10 :10:Chairpersons's remarks

Time	No.	Presentation
10:10-10:30	1	**Fusion activation by parainfluenza virus HN protein** Sayantan Bose, Theodore S. Jardetzky and **Robert A. Lamb**
10:30-10:50	2	**Unraveling a 3-step Mechanism of Nipah Virus Receptor-Induced Membrane FusionTriggering** Qian Liu, Jacqueline A. Stone, Birgit Bradel-Tretheway, Jeffrey Dabundo, Javier A. Benavides, Montano, Jennifer Santos-Montanez, Scott B. Biering1, Xiaonan Lu, **Hector C. Aguilar**
10:50-11 :10	3	**PVRL4 (Nectin-4) is the epithelial cell for measles and canine distemper viruses and is a target for oncolytic therapy** **Christopher Richardson**, Ryan Noyce, SebastienDelpeut, Ricky Siu
11:10-11:20	4	**The measles virus hemagglutininβ4-β5 hydrophobic groove governs the interactions with nectin-4 and CD46 but not with SLAM** **Mathieu Mateo**, Sabriya Syed, ChanakhaNavaratnarajah, Roberto Cattaneo
11:20-11:30	5	**Conversion of the parainfluenza virus 5 F protein to a simian virus 41 HN-specific protein by amino acid substitutions** Talk **Masato Tsurudome** , Morihiro Ito , MachikoNishio, Tetsuya Nosaka
11:30 -12 :30		Coffee break-Poster session
12:30-12:50	6	**Vesiculovirus glycoprotein intermediate structures during the fusion associated conformational change.** Talk Eduard Baquero , Aurélie A. Albertini , MalikaOuldali, Linda Buonocore , John K. Rose , Jean Lepault, Stéphane Bressanelli, **Yves Gaudin**
12:50-13:00	7	**Host cell entry of Respiratory Syncytial Virus involves macropinocytosis followed by proteolytic activation of the F protein** **Magdalena Krzyzaniak**, Ari Helenius
13:00-13:10	8	**Acid-activated structural reorganization of the Rift Valley fever virus Gc fusion protein** Matthijn de Boer, **Jeroen Kortekaas**, LotteSpel, Peter Rottier, Rob Moormann, Berend Jan Bosch'
13:10-13:20	9	**Insights into transport of Marburg virus nucleocapsids in living cells** **Schudt Gordian**, Kolesnikova Larissa, Dolnik Olga, Becker Stephan

13:20-13:30	10	**Essential role of the Sendai virus fusion (F) protein in the formation of virus particles** Manel Essaidi-Laziosi, Anastasia Shevtsova, Carole Bampi, Laurent Roux
13:30-13:40	11	**Orientations of the influenza A viral ribonucleoprotein complexes** Yukihiko Sugita, Hiroshi Sagara, Takeshi Noda, Yoshihiro Kawaoka
13:40-13:50	12	**Diverse Morphologies of Influenza Filament Budding: an Ultrastructural Study** SwethaVijayakrishnan, Colin Loney , Dave Jackson , David Bhella
13:50-15:20		Lunch- Poster session

Poster Presentation

14 **The molecular motor KIF13A mediates intracellular transport of the Lassa virus matrix protein Z**
Sarah Katharina Fehling, Wolfgang Garten, Thomas Strecker
Institute of Virology, Philipps-University Marburg, Germany

15 **The peste des petits ruminants virus (PPRV) receptors: ovine SLAM and Nectin-4**
Jamie Birch 1, Nicholas Juleff 1, Michael P. Heaton 3, Ted Kalbfleisch 4, James Kijas 5 and Dalan Bailey 1,2

16 **Andes Hantavirus Gn and Gc glycoproteins: Self-assembly into virus-like particles and functions during cell entry**
Rodrigo Acuña, Nicolás Cifuentes, Pierre-Yves Lozach, Nicole Tischler

17 **Identification of functional domains of class II Rab11-FIPs that participate in the polarized transport of influenza virus vRNP**
Fumitaka Momose, Takashi Ohkura, Yuko Morikawa

18 **Inhibition of Membrane Fusion by a mAb interacting with the Morbillivirus Attachment Protein Stalk Domain**
Nadine Ader-Ebert1,2, Andreas Zurbriggen1 and Philippe Plattet1

19 **Interaction between M1 and NP affects influenza virus morphology**
Kristy Bialas, Kendra Bussey, Toru Takimoto
Department of Microbiology and Immunology, University of Rochester Medical Center

20 **A Critical Threonine Residue in the Respiratory Syncytial Virus Matrix Protein is required for Matrix Oligomerization and Affect**
Monika Bajorek (1), Sarit Kipper (2), Kim Tran (3), Michael Teng (3), Doron Gerber (2), Reena Ghildyal (4), David Jans (5)

21 **A novel bunyavirus causing severe fever with thrombocytopenia syndrome in humans**
Mifang Liang, Guoyu Liu , Jiandong Li, Lina Sun , Chuan Li, Quanfu Zhang, Cong Jin, Xianjun Wang, Shujun Ding, Zheng Xing, Shiwen Wang, Zhenqiang Bi, Dexin Li

22 **Filamentous influenza virases**
Alex Atkins, Matthew Badham, Jeremy Rossman
School of Biosciences, University of Kent

23 **Respiratory syncytial virus matrix protein: key role in infection**
Reena Ghildyal, Michael N. Teng, John Mills, Philip G. Bardin, David A. Jans

24 **Functional characterization of the surface glycoproteins G and F of an African henipa-like bat virus**
Nadine Krüger, Markus Hoffmann1, Jan-Felix Drexler, Marcel Müller, Michael Weis, Andrea Maisner, Christian Drosten, Georg Herrler

25 **Molecular basis for small molecule inhibitor of Ebola virus infection that targets receptor NPC1**
John Misasi, Anna Bruchez, Marceline Cote, Tao Ren, Kyungae Lee, Soo-mi Lee, James Cunningham

26 **Assembly and budding of Rift Valley Fever virus**
Emily A. Bruce1, Robert W. Doms

27 **Comparative analysis of matrix ubiquitination and nuclear-cytoplasmic trafficking across the paramyxoviridae genera**
Mickey Pentecost , Talia Lester 1, Arnold Park , Yao Wang , Ajay Vashisht, James Wohlschlegel , Benhur Lee

28 **Autophagy pathway plays a critical role in entry of Ebolavirus into host cells**
Olena Shtanko and Robert A. Davey

29 **The impact of GPC and N-terminal region of Lassa virus Z on virus-like particle release**
Shuzo Urata, Jiro Yasuda

30 **Analysis of Borna disease virus transport**
Caroline Marie Charlier, Yuan Ju Wu, Sophie Allart, Cécile Elisabeth Malnou, *Martin Schwemmle, Daniel Gonzalez-Dunia*

31 **The molecular motor KIF13A mediates intracellular transport of the Lassa virus matrix protein Z**
Sarah Katharina Fehling, Wolfgang Garten, Thomas Strecker

32 **Thermodynamics Tune the Paramyxovirus Membrane Fusion Machinery**
Mislay Avila Sánchez, Lisa Alves, Mojtaba Khosravi, Nadine Ader-Ebert, Andreas Zurbriggen and Philippe Plattet

	33	**Studies on fluorescently labeled Nipah virus matrix protein** Boris Lamp, Erik Dietzel, Marc Ringel, Larissa Kolesnikova, Andrea Maisner
	34	**Interaction of Rift Valley Fever virus with the host ESCRT machinery** Amber Riblett, Emily Bruce, Bob Doms
	35	**Endosomal Trafficking of the Nipah Virus Fusion Protein** Michael Weis, Lucie Sauerhering, Sandra Diederich, Andrea Maisner
	36	**Investigating the molecular determinants of mumps virus (MuV) entry and egress** Connor G. G. Bamford , Martin Ludlow, Michael R. Wilson, Linda J. Rennick, Ken Lemon, Bertus K. Rima, W. Paul Duprex
	37	**Temporal analysis of HMPV infection reveals the interplay between viral proteins during the replication cycle** Farah El Najjar, Brent Hackett and Rebecca E. Dutch
	38	**A charge-dependent interaction is necessary for Rift Valley fever virus-like particle entry into its host cell** Maria Baudin, Delowar Hossain, Magnus Evander

Session II: RNA synthesis and gene expression
Chairpersons:Amiya Banerjee and Rob Ruigrok

15:20-15:30:		Chairpersons's remarks
15:30-15:50	39	**An upstream open reading frame modulates ebola virus polymerase translation andvirus replication** Reed S. Shabman, Omar Jabado, **Christopher F. Basler**
15:50-16:10	40	**Initiation and regulation of respiratory syncytial virus transcription and genomereplication** Laure Deflube, Sarah Noton, Chadene Tremaglio, **Rachel Fearns**
16:10-16:30	41	**Functional insights into the measles virus NTAIL-PXD interaction using site-directedand random mutagenesis** Antoine Gruet, Marion Dosnon, Johnny Habchi, David Blocquel, Christophe Bignon, YaolingShu, Joanna Brunel, Michael Oglesbee, Denis Gerlier and **Sonia Longhi**
16:30-16:40	42	**The EM structure of the complementary cRNP replicative intermediate of influenzaA virus and host-pathogen interactions** **Ashley York** , Juha T. Huiskonen , Ervin Fodor
16:40-16:50	43	**The role of the influenza virus nucleoprotein in viral transcription and replication** **L Turrell** , EC Hutchinson , JW Lyall , LS Tiley , E Fodor and FT Vreede

16:50 -17		Coffee break-Poster session
17 :50-18	44	**Influenza nucleoprotein N-terminal deletion mutant is deficient in functional vRNP formation and viral RNA expression.** Abel Sanchez, Christian F. Guerrero-Juarez, Charles C. Peterson, Ryan Laurel, Muriel Makamure, Jose Ramirez, Jacqueline Olivas, and **Laura L. Newcomb**
18 :10-18 :20	45	**Phosphorylation of Ebola virus VP30 influences the composition of the viral nucleocapsid complex: Impact on viral transcription** **Nadine Biedenkopf**, Stephan Becker
18 :20-18 :30	46	**The mechanism of RNA synthesis inhibition by the matrix protein of vesicular stomatitis virus** **Benjamin Morin**, Philip J. Kranzusch, GergelyTekes, Robin A. Ross and Sean P. J. Whelan
18 :30-18 :50	47	**Recoding the L gene of vesicular stomatitis virus by computer-aided synthesis leads to unexpected biological phenotypes** Bingyin Wang , GergelyTekes, Charles Ward, Steve Skiena, Bruce Futcher, Steffen Mueller, Sean Whelan, and **Eckard Wimmer**
19:30		Guided tour of Granada and free evening

Poster Presentation

48	**Functional interaction between PB2 and PA polymerase subunits of influenza virus is a critical determinant of the replication of** Koyu Hara, Yoko Nakazono, Takahito Kashiwagi, Nobuyuki Hamada, Hiroshi Watanabe
49	**The N-terminal fragment of influenza A virus (H5N1) PB2 subunit strongly inhibits its RNA-dependent RNA polymerase** Takahito Kashiwagi*, Koyu Hara, Yoko Nakazono, Yusaku Uemura, Yoshihiro Imamura, Nobuyuki Hamada, Hiroshi Watanabe
50	**Role of non-coding sequences in Arenavirus mRNAs translation** Sabrina Foscaldi, Alejandra D'Antuono, Nora Lopez
51	**Mutations in the mRNA capping enzyme active site of the VSV L protein induce abnormal transcription initiation and termination** Tomoaki Ogino, Amiya K. Banerjee
52	**A novel regulatory mechanism determining the genome polarity of the Mononegavirales** Takashi Irie, Isao Okamoto, Asuka Yoshida, Yoshiyuki Nagai, Takemasa Sakaguchi
53	**Efficient rescue and reverse genetics of viruses from all Paramyxovirinae genera without the use vaccinia-driven T7 polymerase** Shannon M Beaty, Arnold Park, Tatyana Yun, Olivier Pernet, Frederic Vigant, Adria Allen, Yao Wang, Mike Lyons, Nancy L. McQueen, Alexander N Freiberg, Subbiah Elankumaran, Paul Duprex, Benhur Lee

54 **THE ROLES OF PHOSPHORYLATION OF THE MUMPS VIRUS PHOSPHOPROTEIN IN VIRUS REPLICATION**
Adrian Pickar, Pei Xu, James Zengel, Andrew Elson, Zhuo Li, Zhenhai Chen and Biao He

55 **Factors affecting respiratory syncytial virus polymerase activity at the promoters**
Sarah Noton, Laure Deflubé, Chadene Tremaglio, Rachel Fearns

56 **An alternative method to determine the 5' extremities of non-segmented, negative sense RNA viral genomes using positive replicat**
Paul A. Brown, Francois-Xavier Briand, Olivier Guionie, Evelyne Lemaitre, Celine Courtillon, Aurelie Henry, Véronique Jestin and Nicolas Eterradossi

57 **Establishment of a Nipah virus reverse genetics system**
Erik Dietzel, Bevan Sawatsky, Markus Czub, Gary Kobinger, Veronika von Messling, Andrea Maisner

58 **Strategies of avian influenza A virus polymerases for efficient replication after zoonotic transmisión**
Benjamin Mänz, Ron Fouchier

59 **Defective interfering particles hamper continuous influenza virus propagation**
Timo Frensing, Stefan Heldt, Ilona Behrendt, Ingo Jordan , Yvonne Genzel, Udo Reichl

60 **Effect of nuclear export protein (NEP) of influenza virus on regulation of polymerase activity and viral RNA síntesis**
Hongbo Zhou, Anna V. Cauldwell, Olivier Moncorgé, Wendy S. Barclay

61 **Ebola virus: transcriptional RNA editing versus premature poly-adenylation.**
Valentina A. Volchkova, Jaroslav Vorac, Viktor E. Volchkov

62 **Development of a reverse genetics system to generate recombinant Marburg virus derived from a bat isolate**
Luke Uebelhoer, Cesar Albarino, Joel Vincent, Stuart Nichol, Jonathan Towner

63 **Spatiotemporal analysis reveals inclusion bodies as sites of ebolavirus replication**
Thomas Hoenen, Vinod Nair, Reed Shabman, Allison Groseth, Astrid Herwig, Christopher F. Basler, Stephan Becker, Heinz Feldmann

64 **Molecular requirements for inhibiting viral RNA synthesis and host responses by the Nipah virus C protein**, David Karlin, Stuart Nichol, Christina Spiroloulou

65 **Analysis of MARV Viral Genome Non-coding Regions**
Jesus Alonso, Jean L. Patterson

66 **Elucidating the biochemical determinants for restriction of influenza virus polymerase activity by PB2 627E in mammalian cellswered by TCPDF**
Duncan Paterson, Ervin Fodor

67 **Phosphorylation of Marburg virus NP and its influence on replication and assembly**
Anne Brueggemann, Olga Dolnik, Stephan Becker

68 **Involvement of some P residues in the replication complex activity of rabies virus**
Florence Larrous, Olivier Delmas, Alexander Ghanem, Karl Klaus Conzelmann, Hervé Bourhy

69 **Growth kinetic analysis of recombinant measles viruses containing mutations in the phosphorylation sites of nucleoprotein**
Akihiro Sugai, Hiroki Sato, Misako Yoneda, Chieko Kai

70 **Efficient rescue and reverse genetics of viruses from all Paramyxovirinae genera without the use vaccinia-driven T7 polymerase**
Shannon M Beaty, Arnold Park1, Tatyana Yun, Olivier Pernet, Frederic Vigant, Adria Allen, Yao Wang, Michael Lyons, Linda J. Rennick, Nancy L. McQueen, Alexander N Freiberg, Subbiah Elankumaran, W. Paul Duprex, Benhur Lee

Tuesday June 18
Session III:Structure and Function
Chairpersons :Ming Luo and Adolfo Garcia Sastre

10:00-10:10: Chairpersons's remarks

10:10-10:30 71 **Self-organization of the vesicular stomatitis virus nucleocapsid into a bullet shape**
AmbroiseDesfosses, Euripedes A. RibeiroJr, Guy Schoehn, Gregory Effantin, Danielle Blondel, DelphineGuilligay, Marc Jamin, Rob W. H. Ruigrok and **Irina Gutsche**

10:30-10:50 72 **The crystal structure and RNA-binding of an orthomyxovirus nucleoprotein**
WenjieZheng , John Olson , Vikram Vakharia , **Yizhi Jane Tao**

10:50-11:10 73 **Functional and structural studioes on Schmallenberg virus, a newly emerged orthobunyavirus in Europe**
Richard M. Elliott , Gjon Blakqori ,Ingeborg C. van Knippenberg, ElinaKoudriakova , Ping Li , Angela McLees , Xiaohong Shi , Agnieszka M. Szemiel , Haohao Dong and Changjiang Dong

11:10-11:20 74 **Structural basis for encapsidation of genomic RNA by La Crosse Orthobunyavirus nucleoprotein**
Juan Reguera, Hélène Malet, Friedemann Weber and Stephen Cusack

11:20-11:30	75	**Dissecting the Multifunctional Nucleoprotein of Arenaviruses** **Emilio Ortiz-Riaño**, Benson Y.H. Cheng, Juan C. de la Torre, Luis Martínez-Sobrido
11:30 -12 :30		Coffee break-Poster session
12:30-12:50	76	**Structural analyses of influenza A virus polymerase and RNPs** RocíoArranz, Rocío Coloma, Patricia Resa-Infante, NoeliaZamarreño, Maria Angeles Recuero-Checa, Roberto Melero, José M. Valpuesta, José L. Carrascosa, Oscar Llorca, Jaime Martín-Benito and **Juan Ortín**
12:50-13:10	77	**Biochemical and biophysical analysis of disctinctive human resiratory syncytial virus proteins: phospoprotein, M2-1 antiterminat** Sebastián Esperante, Esteban Pretel and **Gonzalo de Prat Gay**, Fundación Instituto Leloir. IIBBA CONICET. Buenos Aires, Argentina
13:10-13:30	78	**A polymerase supercomplex within vesicular stomatitis virus** Jeffery Hodges, Xiaolin Tang, Michael B. Landesman, John B. Ruedas, Anil Ghimire, Manasa V. Gudheti(, Jacques Perrault, Erik M. Jorgensen, Jordan M. Gerton and **SaveezSaffarian**
13:30-13:50	79	**Crystal structure of the N0-P complex of Nipah virus and of VSV provide new insights into the encapsidation mechanism** FilipYabukarski, CédricLeyrat, Nicolas Tarbouriech, MaleneRingkjøbing Jensen, Martin. Blackledge, Rob Ruigrok1 and **Marc Jamin**
13:50-15:20		Lunch- Poster session

Poster Presentation

	80	**Functional insights into measles virus NTAIL-PXD interaction using the biGene/biSilencing (biG/BiS) system** Joanna Brunel, Antoine Gruet, Marion Dosnon, Patricia Devaux, Roberto Cattaneo, Sonia Longhi et Denis Gerlier
	81	**A STRUCTURE FUNCTION ANALYSIS OF THE SENDAI VIRUS MATRIXPROTEIN USING AN INTEGRATED SUPPRESSION COMPLEMENTATION SYTEM (ISCS)** Geneviève Mottet-Osman and Laurent Roux
	82	**Isolation and characterization of an N°-P complex of the Respiratory Syncytial Virus** Marie Galloux, Gaëlle Gabiane, Jean-François Eléouët

83	**Amino acid substitutions in human respiratory syncytial virus N protein affect differentially viral transcription and replication** Ana Asenjo, Maria Luisa Navarro and Nieves Villanueva
84	**Labeling of influenza viruses with synthetic fluorescent and biotin-labeled lipids** Natalia A. Ilyushina, Evgeny S. Chernyy, Elena Y. Korchagina, Aleksandra S. Gambaryan, Stephen M. Henry, Peter F. Wright , Nicolai V. Bovin
85	**Disulphide-linked CM2 oligomers are required for efficient replication of influenza C virus** Yasushi Muraki, Takako Okuwa, Toshiki Himeda, Seiji Hongo, Yoshiro Ohara
86	**Structure of the mumps virus phosphoprotein** Robert Cox, Todd J. Green, Sangeetha , Champion Deivanayagam, Gregory J. Bedwell, Peter E. Prevelige and Ming Luo
87	**Location and structure of an antibody-binding site of the body domain of influenza A virus nucleoprotein** Natalia Varich, Galina Sadykova, Prilipov Alexey, Kochergin-Nikitsky Konstantin, Kaverin Nikolai
88	**Influenza virus non-structural protein NS1 cooperatively binds virus-specific (+)-strand RNA sequences** Daniel MARC, Denis Soubieux
89	**NMR reveals alpha-helical propensity in RSV P protein outside the oligomerization domain** Safa Lassoued, Marie Galloux, Jenna Fix2, Carine Van Heijenoort, François Bontems, Jean-François Eléouët, Christina Sizun
90	**Characterization of the multiple Crimean-Congo Hemorrhagic Fever Virus Nucleoprotein interactions** Jesica M. Levingston Mac leod, Natalia Frías-Staheli and Adolfo García-Sastre
91	**NiV N expression is repressed by hnRNP D wih its binding to NiV N 3'UTR** Kimihiro Hino, Hiroki Sato, Akihiro Sugai, Masahiko Kato, Misako Yoneda, Chieko Kai
92	**Interactions between the Sendai virus HN glycoprotein and the matrix M protein** Anastasia Shevtsova, Manel Essaidi-Laziosi, Laurent Roux
94	**The Cys3-His1 Zinc binding motif of the hRSV M2-1 tetramer modulates its dissociation to folded apo-monomers.** Sebastian Esperante, Maria Gabriela Noval, Tamara Antonella, and Gonzalo de Prat Gay

	95	**The Measles Virus Nucleoprotein Tail is Required for Packaging of Viral Polymerase Components into Nascent Particles** Stefanie Krumm, Richard Plemper
	96	**Folding and conformational diversity of the non-structural NS1 protein from hRSV.** Esteban Pretel, Mariana Gallo, Gabriela Camporeale and Gonzalo de Prat Gay
	97	**Probing conformational changes of the RSV F protein using conformation-specific antibodies and recombinant soluble F proteins** Alan Rigter, Ivy Widjaja, Peter J.M. Rottier, Bert Jan Haijema, Cornelis A.M. de Haan
	98	**Structural characterization of measles virus P oligomerization domain** David Blocquel, Johnny Habchi, Eric Durand, François Ferron, Nicolas Papageorgiou and Sonia Longhi
	99	**The N-terminus of NEP of Influenza A viruses regulates the polymerase-enhancing function by backfolding to the C-terminu** Peter Reuther, Linda Brunotte, Veronika Götz, Martin Schwemmle

programe

Session IV:Host-cell interactions
Chairpersons:Denis Gerlierand Peter Staehli

15:20-15:30: Chairpersons's remarks

15:30-15:50	100	**Lyssavirus targeting of STAT proteins: a critical determinant of pathogenicity and potential therapeutic target** Linda Wiltzer , Aaron Brice , Satoko Yamaoka , Florence Larrous, Herve Bourhy , Danielle Blondel , David Jans , Naoto Ito , **Gregory Moseley**
15:50-16:10	101	**Marburg virus structural protein VP24 acts as an activator of Nrf2 pathway by targeting cellular Keap1.** A.Page, SP.Reid, M.Mateo, K.Nemirov, V.A.Volchkova, A.C.Shurtleff, P.Lawrence, A.Baule, O.Reynard, M.Ottmann, V.Lotteau, R.K.Thimmulappa, S.Biswal, S.Bavari, **V.E.Volchkov**
16:10-16:20	102	**Identification of an Endocytic Calcium Channel TPC2 as a Novel Host Factor of Ebolavirus Infection** **YasuteruSakarai**, Andrey A. Kolokoltsov, Robert A. Davey
16:20-16:40	103	**Epigenetic Factors in Arenavirus Infection: Roles for Glycosylation andPhosphorylation** **Michael Buchmeier,** Cyrille Bonhomme, Kristeene Knoop, Lydia Bederka

16:40-16:50	104	**The Intracellular Cargo Receptor ERGIC-53 is Required for the Production of Infectious Arenavirus Particles** **Joseph P. Klaus** , Philip Eisenhauer, Joanne Russo, Anne Mason, Danh Do1, Benjamin King, Douglas Taatjes, Cromwell Cornillez-Ty, Jonathan Boyson, ChunleiZheng, Lujian Liao, John R. Yates III, Bin Zhang, Bryan A. Ballif, and Jason Botten
16:50 -17 :50		Coffee break-Poster session
17:50-18:00	105	**Deciphering the glycosylome of dystroglycanopathies with a haploid genetic screen for Lassa virus cell entry** **MatthijsRaaben**, Lucas T. Jae, MoniekRiemersma, Ellen van Beusekom, Vincent A. Blomen, Arno Velds, Ron. M. Kerkhoven, Jan E. Carette, HalukTopaloglu, Peter Meinecke, Marja W. Wessels, Dirk J. Lefeber, Hans van Bokhoven, Thijn R. Brummelkamp and Sean P. Whelan
18:00-18:10	106	**Nairobi Sheep Disease Virus (NSDV) blocks the JAK-STAT-signalling pathway at the level of the Janus kinases** **Barbara Holzer**, Michael D Baron
18:10-18:20	107	**Analysis of the NSs protein of the newly emerged Schmallenberg virus** **Gerald Barry** , Mariana Varela , Marco Caporale , FraukeSeehusen, MaximeRatinier , Esther Schnettler, Ilaria M. Piras , Melanie McFarlane , Wolfgang Baumgärtner , Alain Kohl, Massimo Palmarini
18:20-18:40	108	**Fragile X Mental Retardation Protein Stimulates Ribonucleoprotein Assembly of Influenza A Virus** Zhuo Zhou, Yang Guo, Lili Zhao, Jingfeng Wang1, Xue Jia Qinghua Xue, Sheng Cui, Qi Jin, Jianwei Wang, **Tao Deng**
18:40-18:50’	109	**The Splicing Factor Proline-Glutamine Rich (SFPQ/PSF) Is essential in Influenza Virus Polyadenilation** **Sara Landeras-Bueno**, Núria Jorba, Maite Pérez-Cidoncha and Juan Ortín
18:50-19:00	110	**Viral proteins and RIG-I like receptor interaction** **Maria T Sanchez-Aparicio**, Juan Ayllon, Adolfo Garcia-Sastre
19:00-19:10	111	**B-1 is a porter to lead influenza viral ribonucleoprotein complexes to microtubules** **Atsushi KAWAGUCHI,**Kyosuke NAGATA
20:00	Alhambra’s visit + dinner in the gardens overlooking Granada by night	

Poster Presentation

	112	**Interaction between human parainfluenza virus type 2 V protein and an antiviral host factor, tetherin** Machiko Nishio, Keisuke Ohta, Masato Tsurudome, Daniel Kolakof-sky

113 **Extrinsic apoptosis subversion by RSV P in macrophages**
Yuko Nakamura-López, Nicolás Villegas-Sepulveda , Beatriz Goméz

113.a **Paradoxical effect of nitric oxide on respiratory syncytial virus replication in a persistently infected murine macrophage cell**
Kenya M. Maldonado-Pérez, Carlos Santiago-Olivares, Beatriz Gómez

114 **Tracking of fluorescently labeled Newcastle Disease Viruses in living cells**
Angela Römer-Oberdörfer, Anja Röder, Stefan Finke, and Thomas C. Mettenleiter

115 **Hsp70 protein positively regulates Rabies Virus infection**
Xavier Lahaye, Aurore Vidy, Baptiste Fouquet, Danielle Blondel

116 **CELL-SURFACE RECEPTOR USAGE OF OCOZOCOAUTLA DE ESPINOSA VIRUS**
Yíngyún Caì, Steven Mazur1, Shuǐqìng Yú1, Lián D ng, Krisztina Janosko1, Téngfēi Zhāng, Marcel A. Müller, Sina Bavari, Peter B. Jahrling, Sheli R. Radoshitzky, and Jens H. Kuhn

117 **Deciphering the antiviral activity of the human Mx system in a transgenic mouse model**
Christoph Deeg, Pascal Mutz, Carsten Kallfass, Cindy Nürnberger, Sébastien Soubies and Peter Staeheli

118 **The host-cell transcription machinery is a target for influenza virus polymerase-induced degradation that contributes to viral**
A. Rodriguez, R. Alfonso, C. Llompart & A. Nieto

119 **Phospholipase (PLC) gamma 1 signaling plays a subtype-specific role in cell entry of influenza A virus**
Liqian Zhu, Vikram Verma, Naveen Kumar, Nishi R. Sharma, and Yuying Liang
Department of Veterinary and Biomedical Sciences, University of Minnesota, Twin Cities, MN 55108. USA.

120 **Isolation and Characterization of Hokkaido Virus, Genus Hantavirus**
Takahiro Sanada1, Yuka Ozaki, Takahiro Seto, Momoko Nakao, Saasa Ngonda, Kumiko Yoshimatsu2, Jiro Arikawa, Kentaro Yoshii, Ikuo Takashima and Hiroaki Kariwa

121 **Relative cytopathogenesis of RSV in nasal epithelial cell cultures from infant cohorts with histories of severe and mild RSV.**
Hong Guo-Parke, Olivier Touzelet, Isobel Douglas, Rémi Villenave1, Liam G. Heaney, Peter V. Coyle, Michael D. Shields and Ultan F. Power

122 **ANP32B is a Nuclear Target of Hendra Virus Matrix Protein**
Sebastian Neumann, Axel Karger, Ann-Kristin Henning, Linda Kwasnitschka, Anne Balkema-Buschmann, Günther Keil, Stefan Finke

123 **High-content siRNA screen to identify cellular factors required for infection of Crimean-Congo hemorrhagic fever virus**
Olena Shtanko,Raisa Nikitina,Alexander Chepurnov, Robert Davey

124 **Host mTORC1 signaling regulates Andes virus replication**
Shannon McNulty, Michael Flint, Stuart Nichol, Christina Spiropoulou

125 **Differential recognition of arenavirus glycoproteins and cellular substrates by subtilisin kexin isozyme 1/site 1 protease**
Dominique J. Burri, Joel Ramos da Palma, Nabil G. Seidah, Giuseppe Zanotti, Laura Cendron, Antonella Pasquat1, and Stefan Kunz

126 **Viral dsRNA detection by the RIG-I Like Receptors family**
Jessica Guerra, Stéphanie Anchisi, Dominique Garcin

127 **Dissecting the molecular interaction of influenza A virus RNA polymerase and host RNA polymerase II.**
Mónica Martínez-Alonso, Narin Hengrung, Lauren Turrell,

128 **African Henipavirus surface proteins promote viral entry, cell fusion & cytopathic effects in Human, Simian & Bat cell lines**
Philip Lawrence, Jan-Felix Drexler, Victor Max Corman, Beatriz Escudero-Perez1, Valentina Volchkova, Marcel Müller, Christian Drosten & Viktor Volchkov

129 **Mutual Antagonism between Ebola virus VP35 and PACT**
Priya Luthra, Parameshwaran Ramanan, Chad Mire, Carla Weisend, Yoshimi Tsuda, Benjamín Yen, Daisy W. Leung, Thomas W. Geisbert, Hideki Ebihara, Gaya. K. Amarasinghe, Christopher F. Basler

130 **Residue 57 of the Marburg Virus VP40 Matrix Protein Determines Resistance to Human Tetherin**
Alicia Feagins, Christopher Basler

131 **Borna disease virus X protein: a new tool against neurodegenerative disorders?**
Marion Szelechowski , Alexandre Bétourné, Yann Monnet, Anne Thouard, Jean-Michel Peyrin, Stéphane Hunot, Daniel Gonzalez-Dunia

132 **Length of the NS1 linker region determines pathogenicity of a highly pathogenic H5N1 influenza A virus**
Mirco Schmolke, Benjamin G Hale, Rong Hai, Jianqiang Ye, Balaji Manicassamy, Daniel R Perez, Adolfo García Sastre

133 **The tetraspanin proteins, CD81 and CD9, promote efficient influenza A virus budding through interactions with HA and NA**
Yuhong Liang, Megan L. Shaw

134 **Importance of the P2 and P3 residues of fusion proteins of respiratory viruses for their proteolytic activation by TMPRSS2**
Maino Tahara, Masako Abe, Kouji Sakai, Kazuya Shirato, Kazuhiko Kanou1, Shutoku Matsuyama1, Hideo Fukuhara, Katsumi Maenaka, Yasushi Ami, Mariko Esumi, Atsushi Kato, Makoto Takeda

135 **Influenza A virus NS1 and PI3K: strain and isotype specificity of a complex virus-host interaction**
Ayllon Juan, Hale Benjamin G., Sánchez-Aparicio María T., García-Sastre Adolfo

136 **Modulatory effect of heat shock protein 70 (Hsp70) on influenza A virus polymerase activity**
Rashid Manzoor, Kazumichi Kuroda, Reiko Yoshida, Yoshimi Tsuda, Daisuke Fujikura, Hiroko Miyamoto, Masahiro Kajihara, Hiroshi Kida, Ayato Takada

137 **The importin-α7 gene is a determinant of influenza virus cell tropism in the murine lung**
Patricia Resa-Infante, René Thieme, Petra Arck, Rudolph Reimer, Gülsah Gabriel

138 **Characterization of influenza virus receptors in the respiratory tract of ferrets**
Miranda de Graaf, Angela van Diepen, Monique I. Spronken, Sander Herfst, Corneel H. Smit, Albert D.M.E. Osterhaus, Derek J. Smith, Corneel H. Hokke, Ron A.M. Fouchier

139 **Novel host factors that promote influenza A virus uncoating**
Yohei Yamauchi, Ari Helenius

140 **Identification of host factors interacting Borna disease virus ribonucleoprotein in the nucleus**
Tomoyuki Honda, Akiko Makino, Kan Fujino, Kozue Sofuku, Shoko Nakamura, Keizo Tomonaga

141 **Analysis of cell entry of a novel arenavirus, Lujo virus, using pseudotype VSV**
Hideki Tani, Masayuki Shimojima, Shuetsu Fukushi, Tomoki Yoshikawa, Masayuki Saijo, and Shigeru Morikawa

142 **Role of cholesterol transporter proteins NPC1 and NPC1L1 in Lujo virus entry and replication.**
Punya Shrivastava-Ranjan, Ayan K Chakraborti, Stuart T Nichol, Chrsitina F Spiropoulou

143 **Cellular adaptation for establishment of measles virus persistent infection in culture**
TOMOMITSU DOI, HIROKI SATO, MISAKO YONEDA, CHIEKO KAI

144 **DDX1 and DDX3 as potential host factors affecting influenza virus replication in Swine Respiratory Epithelial cells (SRECs)**
Sathya Narayanan Thulasi Raman, Yan Zhou

145 **Influenza A virus protein PB1-F2 reduces viral pathogenesis in chicken**
Olivier Leymarie , Hana Weingartl, Bernard Delmas,Ronan Le Goffic

146 **Characterization of the role of human cathepsin W in the entry process of influenza A virus**
Thomas Edinger, Marie-Theres Pohl, Silke Stertz

147 **Primary airway epithelial cells from swine provide a model system to study proteolytic activation of influenza virases**
C. Peitsch, H.- D. Klenk, W. Garten, E. Böttcher-Friebertshäuser

148 **The regulation and functional significance of GALNT3 expression during influenza A virus infection**
Shoko Nakamura, Masayuki Horie, Mayo Yasugi, Daisuke Okuzaki, Akiko Makino, Tomoyuki Honda, Keizo Tomonaga

149 **Nairobi sheep disease virus causes translocation of ER chaperones in infected cells**
Lidia Lasecka, Haru-Hisa Takamatsu, Dirk Werling, Michael D Baron

150 **Junin virus NP but not Tacaribe virus NP serves a decoy-function to prevent apoptosis induction during infection**
Svenja Wolff, Allison Groseth, Stephan Becker

151 **Prolidase (PEPD) is a required human factor for influenza A virus entry**
Marie-Theres Pohl, Thomas Edinger, Silke Stertz

152 **Host-dependence of the thermosensibility of measles virus vaccine strains and critical function of HSP90 in viral replication**
Louis-Marie Bloyet, Jérémy Welsch, Jessica Rabilloud, Branka Horvat, Denis Gerlier, Boyan Grigorov.

153 **Novel insights into the divergent mechanisms of paramyxovirus immune evasion**
Michelle Audsley, Glenn Marsh, Mary Tachedjian, Linfa Wang , David Jans, and Gregory Moseley.

Wednesday June 19
09:00-10:00Put up posters for sessions V-VIII

Session V: Antiviral response

Chairpersons : Klaus Conzelmann andDanielle Blondel

10:00-10:10: Chair persons's remarks

10:10-10:20	154	**Paramyxovirus V proteins disrupt the fold of the innate immune sensor MDA5 to inhibit antiviral interferon response** Carina Motz, Kerstin Monika Schuhmann , Axel Kirchhofer , Manuela Moldt , Gregor Witte , Karl-Klaus Conzelmann , Karl-Peter Hopfner
10:20-10:30	155	**Paramyxovirus V proteins interact with the TRIM25/RIG-I regulatory complex and inhibit RIG-I signaling** Leighland J. Feinman, Maria T. Sanchez-Aparicio , Adolfo García-Sastre, Megan L. Shaw
10:30-10:40	156	**Molecular basis of RIG-I activation** Stéphanie Anchisi, Jessica Guerra, Dominique Garcin
10:40-10:50	157	**Unanchored Lysine48-linked polyubiquitin chains positively regulate the type I IFN-mediated antiviral response** Ricardo Rajsbaum, Gijs A. Versteeg, Sonja Schmid, Ana M. Maestre, AlanBelicha-Villanueva, Jenish R. Patel, Juliet Morrison, Giuseppe Pisanelli, AnaFernandez-Sesma, Benjamin R. tenOever, Adolfo García-Sastre
10:50-11:10	158	**STAT2 deficiency and susceptibility to (NSV) viral illness in humans** ophieHambleton, Stephen Goodbourn, Dan F. Young, Paul Dickinson, Siti M.B. Mohamad, ManojValappil, Naomi McGovern, Andrew J. Cant , Scott Hackett, Peter Ghazal, Neil V. Morgan&Richard E. Randall
11:10-11:20	150	**Differences in interferon β inhibition between the C proteins of measles virus vaccine and wildtype strains** Konstantin Sparrer , Christian K. Pfaller , Karl-Klaus Conzelmann
11:20-11:30	160	**The matrix protein of rabies virus binds to RelAp43 to suppress NF-κ-B dependent gene expression related to innate immunity** Youcef Ben Khalifa , Sophie Luco , Mehdi Archambaud , Olivier Delmas , Jonathan Grimes, Hervé Bourhy
11:30 -12 :30		Coffee break-Poster session
12:30-12:50	161	**Sequestration of RNA-regulating proteins in Ebola virus inclusions** Kristina Maria Schmidt , Emily Nelson Judith Olejnik , Kristina Brauburger , Travis Taylor , Logan Banadyga , Hideki Ebihara , ElkeMühlberger

12:50-13:00	162	**Ebola Virus Secreted Glycoprotein Alters the Host Antibody Response via "Antigenic Subversion"** **<u>Gopi S. Mohan</u>**, Ling Ye, Chinglai Yang, Richard W. Compans
13:00-13:10	163	**Respiratory syncytial virus (RSV) NS1 protein inhibits host cell apoptosis mediated by the interferon induced transmembraneprot** **<u>Shirin Munir</u>**, Peter L. Collins
13:10-13:20	164	**Utilization of host ubiquitin ligases by Rift Valley fever virus virulence factor NSs** **<u>Markus Kainulainen</u>** , Matthias Habjan, Laura Busch , Philipp Hubel , Simone Lau , Andreas Pichlmair , GiulioSuperti-Furga, Friedemann Weber
13.20-13 :30	165	**Interferon-inducible Mx1 protein inhibits influenza virus by interfering with viral ribonucleoprotein complex assembly** **<u>Judith Verhelst</u>** , EefParthoens , Bert Schepens , and Xavier Saelens
13 :30-13 :50	166	**Pandemic influenza A viruses escape from restriction by human MxA through adaptive mutations in the nucleoprotein** Benjamin Mänz , Dominik Dornfeld , Veronika Götz , Roland Zell , Petra Zimmermann , Otto Haller , Georg Kochs and **<u>Martin Schwemmle</u>**
13:50-15:20		Lunch- Poster session

programe

Poster Presentations:

	167	**Chicken MDA5 senses short double-stranded RNA and is involved in interferon induction against influenza virus infection** Takehiko Saito, Tsuyoshi Hayashi, Chiaki Watanabe, Yasushi Suzuki, Taichiro Tanikawa, Yuko Uchida
	168	**Humoral response at immunization with live and inactivated EBOV** Alexander Chepurnov
	169	**Degradation of RIG-I and inhibition of IFN-beta induction by Toscana Virus NSs protein.** Maria Grazia Cusi, Gianni Gori Savellini, Chiara Terrosi, Barbara Martorelli
	170	**Cytokine profil in Crimean-Congo Hemorrhagic fever** Anna Papa, Katerina Tsergouli, Dilek Yağcı Çağlayık, Gulay Korukluoglu, Yavuz Uyar, Najada Como, Majlinda Kota, Eugena Tomini, Silvia Bino

171 **A Comprehensive Proteomic View of the Suppression of Host Cell Antiviral Responses by Respiratory Syncytial Virus**
Keyur Dave1, Alexander Bukreyev, Emma Norris1, Ursula Buchholz, Marcus Hastie, Madeleine Headlam, Buddhika Jayakody, Kirsten Spann, Cassandra Wright, Toshna Singh, Peter Collins and Jeffrey Gorman

172 **Influenza virus infection induced Ebp1 modification**
Ayae Honda, Shinichiro Kume, Yukari Miyake, Koji Kadoi

173 **Intrinsic and innate immune properties of PMLIV during VSV infection**
Faten El Asmi, Mohamed Ali Maroui, Nathalie Roders, Danielle Blondel, Sébastien Nisole, and Mounira K. Chelbi-Alix

174 **Antigen editing by monocyte-derived dendritic cells in the lung shapes the quality of anti-influenza T cell immunity**
Jazmina L.G. Cruz, Anja Lüdtke, Sergio Gómez-Medina, José V. Pérez-Girón and César Muñoz-Fontela

175 **Both the C and V proteins of canine distemper virus play essential roles for the virus replication in human epithelial cells**
Noriyuki Otsuki, Tsuyoshi Sekizuka, Toru Kubota, Yuichiro Nakatsu, Fumio Seki, Kouji Sakai, Ryoji Yamaguchi, Hideo Fukuhara, Katsumi Maenaka, Makoto Kuroda, Makoto Takeda

176 **Responses to Pneumococcus in HumangInfluenza infection Directly Alters Innate IL-23 and IL-12p70 and Subsequent IL-17A and IFN-**
Sinead Loughran*, 1Patrick Power*,Samantha McQuaid, Ingileif Jonsdottir, Richard Lalor and Patricia A Johnson

177 **Prevalence and predictors for heterosubtypic antibodies against influenza A in Humans**
Ines Kohler, Alexandra U. Scherer, Osvaldo Zagordi, Arkadiusz Wyrzucki, Matteo Bianchi, Marco Steck, Bruno Ledergerber, Huldrych F. Günthard and Lars Hangartner

178 **Inhibition of Borna disease virus replication by an endogenous bornavirus element in ground squirrel genome**
Kan Fujino, Masayuki Horie, Tomoyuki Honda, Keizo Tomonaga

179 **Systematic Identification and Characterization of Interferon-Induced Antiviral Factors**
Yao Wang, Su-Yang Liu, Roghiyh Aliyari, Genhong Cheng

180 **Interferon (IFN) induces an antiviral state in cells that results in alterations of the patterns**
Jelena Andrejeva, Hanna Norsted, Stephen Goodbourn, Rick Randall

181 **Interactions between Natural Killer cells and antigen presenting cells during the infection by Lassa virus**
Marion Russier, Stéphanie Reynard, Sylvain Baize

182 **Variation in the interferon response induced by influenza A virus strains.**
Konrad Bradley, Lorian Hartgroves, Ruth Elderfield, Holly Shelton and Wendy Barclay.

183 **Deep sequencing analysis of defective genomes of parainfluenza virus 5 and their role in interferon induction.**
Marian Killip, Dan Young, Derek Gatherer, Craig Ross, Andrew Davison, Steve Goodbourn, Richard Randall

184 **RVFV growth in human macrophages is severely restricted by knockout of a PSL motif in the virus NSs virulence factor**
Anita K McElroy, Kimberly A Dodd, Stuart T Nichol and Christina F Spiropoulou

185 **Interferon-inducible antiviral protein Tetherin/BST-2 inhibits Hazara virus Replication**
Yohei Kurosaki, Akiko Nishimura, Shuzo Urata and Jiro Yasuda

186 **Interaction between human parainfluenza virus type 2 V protein and tetherin, an antiviral host factor**
Keisuke Ohta, Hideo Goto, Natsuko Yumine, Machiko Nishio

187 **Inhibition of NF-κB activation by peptide derived from nucleoprotein of Borna disease virus**
Akiko Makino, Kan Fujino, Yuya Hirai, Kozue Sofuku, Shoko Nakamura, Shohei Kojima, Tomoyuki Honda, Keizo Tomonaga

188 **The never-ending battle between the Innate Immune System and the viruses: Influenza A NS1 protein**
Jessica Guerra, Stéphanie Anchisi, Dominique Garcin

189 **Mononegavirales leader RNA as agonist of RIG-I**
LOUBER Jade, BLOYET Louis-Marie, KOWALINSKI Eva, CUSACK Stephen, GERLIER Denis

190 **Different production of a viral RNA species between Sendai virus strains causes their remarkable difference in IFN inducibility**
Asuka Yoshida, Ryoko Kawabata, Takemasa Sakaguchi, Takashi Irie

191 **Measles Virus Neutralization: Genotype-dependent neutralization epitopes in the Hemagglutinin protein which are immunogenic in natural infection and vaccination and elicit a long-term B cell memory.**
Miguel Ángel Muñoz-Alía, César Santiago, José M. Casasnovas, María L. Celma y Rafaél Fernández-Muñoz

192 **PPRV, The role of its accessory proteins in the inhibition of the induction of IFNβ**
Beatriz Sanz Bernardo, Stephen Goodbourn, Michael D Baron

193 **Respiratory syncytial virus induces but antagonises innate antiviral responses in well-differentiated paediatric primary bronchi**
Rémi Villenave, Lindsay Broadbent, Isobel Douglas, Jeremy D. Lyons, Peter V. Coyle, Michael N. Teng, Ralph A. Tripp, Liam G. Heaney, Michael D. Shields, Ultan F. Power

194 **An UNBIASSED GENETIC SCREEN REVEALS the POLYGENIC NATURE of the INFLUENZA VIRUS ANTI-INTERFERON RESPONSE**
Maite Pérez-Cidoncha, Marian J. Killip, Juan C. Oliveros, Víctor Asensio, José A. Bengoechea, Richard E. Randall, Juan Ortín

195 **Characterizing the antiviral response of the Pteropus vampyrus bat, an important ecological reservoir of henipaviruses**
Nicole B Glenno, Omar Jabado, Michael K Lo, Megan L Shaw

196 **Rabies virus P-protein interaction with STAT proteins is critical to lethal rabies Disease**
L. Wiltzer, S. Yamaoka (3), F. Larrous, H.V. Kuusisto , D. Blondel, A. Brice, H. Bourhy, D.A. Jans, N. Ito and G.W. Moseley

197 **Determinants for dsRNA-binding and PKR inhibition in the NS1 protein of influenza A virus as revealed by reverse genetics**
Kristina L. Schierhorn, Katrin Hoegner, Julia Dzieciolowski, Susanne Herold, Stephan Pleschka, Thorsten Wolff1

198 **ISG15 and its role in the restriction of influenza A virus host range**
Jessica Knepper, Viola K. Weinheimer, Thorsten Wolff

Session VI: Pathogenesis

Chairpersons:Otto Haller and Yoshi Kawaoka

15:20-15:30 Chairpersons's remarks

15:30-15:50 199 **Impact of borna disease virus persistence on neuronal homeostasis**
AlexandreBétourné, Marion Szelechowski, Emilie Bonnaud, Anne Thouard, Charlotte Foret, **Daniel Gonzalez-Dunia,** Cécile E. Malnou

15:50-16:10 200 **Cedar virus: A novel henipavirus isolated from Australian bats**
Glenn A Marsh, Lin-Fa Wang

16:10-16:20 201 **Soluble Proteins Of Ebola Virus Activate Dendritic Cells And Macrophages Causing Release Of Pro- And Anti-Inflammatory Cytokines**
Beatriz Escudero Pérez, Philip Lawrence and Viktor Volchkov

16:20-16:30 202 **Human Metapneumovirus SH and G glycoproteins Inhibit Macropinocytosis-Mediated Entry Into Dendritic Cells and Reduce CD4+ T Cell**
Cyril Le Nouën, PhilippaHillyer , Linda G Brock , Christine C Winter , Ronald L Rabin , Peter L Collins , and Ursula J Buchholz

16:30-16:40	203	**Wild Type Measles Virus Infection Up-Regulates PVRL4 and Causes Apoptosis inBrain Endothelial Cells by Induction of TRAIL** Hani'ah, Abdullah , Brenda Brankin , Clare Brady , S**. Louise Cosby**
16:40-16:50	204	**Furin cleavage and in vivo imaging of Crimean-Congo hemorrhagic fever virus secreted non-structural glycoproteins** Éric **Bergeron**, Dennis A. Bente, Ayan K. Chakrabarti, Alexander McAuley, Jessica R. Spengler, Stuart T. Nichol, César G. Albariño, Christina F. Spiropoulou
16:50-17 :00	205	**Contribution of the NSm proteins of Rift Valley Fever virus to virus propagation and virulence in mammalian and arthropod hosts** Felix Kreher, Carole Tamietti , Celine Gommet , Laurent Guille-mot , Anna-Bella Failloux , Jean Jacques Panthier , Michèle Bou-loy , Marie Flamand
17:00 -18:00		Coffee break-Poster session
18:00-18:10	206	**Hantavirus infection confers resistance to NK cell-mediated killing and hantavirus N protein inhibits gzmB and caspase 3** **Shawon Gupta**, Monika Braun, Nicole Tischler, Malin Stoltz, Karin Sundström, Niklas Björkström
18:10-18:30	207	**The hemagglutinin, nucleoprotein and neuraminidase gene segments contribute to virulence in a chimeric H4/H5 avian influenza virus** JuttaVeits, Jana Hundt, Siegfried Weber, Jürgen Stech and **Thomas C. Mettenleiter,**Institute of Molecular Biology, Friedrich
18:30-18:40	208	**Mitochondrial Targeting Sequence Mutations In PB1-F2 Protein Enhance The Virulence of Pandemic H1N1 Influenza A Virus** **Subbiah Elankumaran**, JagadeeswaranDeventhiran, Sandeep RP Kumar
18:40-18:50	209	**Newly identified motifs of influenza A protein PB1-F2 that prime viral and secondary bacterial pneumonia** **Irina Alymova**, Amali Samarasinghe, Amanda Green, Peter Vogel, Jon McCullers
18:50-19:00	210	**A Systems Biology Approach Reveals Novel Host Response Genes that Differentially Regulate Influenza Virus Pathogenicity** **Amie J. Eisfeld** , Hugh Mitchell , Michael G. Katze , Katrina M. Waters , and Yoshihiro Kawaoka Free evening

211 **Efficient induction of viral encephalitis after aerosol exposure to Rift Valley Fever Virus**
Amy L. Hartman, Diana S. Powell, Laura M. Bethel, Amy L. Caroline, Jacquelyn M. Bales, Richard J. Schmid, Timothy D. Oury, and Douglas S. Reed

212 **Heterologous exchanges of non-virion protein and glycoprotein in Novirhabdoviruses:effects on pathogenicity and host specificity**
Arun Ammayappan, Gael Kurath, Tarin Thompson, Shamila Yusuff, Vikram Vakharia

213 **Experimental infection of rhesus and cynomolgus macaques with a wild water bird-derived highly pathogenic avian influenza virus**
Tomoko Fujiyuki, Misako Yoneda, Fumihiko Yasui, Takeshi Kuraishi, Shosaku Hattori, Hyun-jeong Kwon, Keisuike Munekata, Yuri Kiso, Hiroshi Kida, Michinori Kohara, Chieko Kai

214 **Symptomatic highly pathogenic avian influenza virus restores pathogenicity in chicken by serial egg pasajes**
Yuko Uchida, Yasushi Suzuki, Takehiko Saito

215 **The incubation period is longer than the latent period in chickens and turkeys experimentally infected with Highly Pathogenic Av**
James Seekings, Chad Fuller, Caroline Warren, June Mynn, Ross Cooper, Elizabeth Aldous, Richard M. Irvine and Ian H. Brow

216 **Pathogenesis of infection of pregnant gilts with H1N2 swine influenza virus**
Iwona Markowska-Daniel, Krzysztof Kwit, Małgorzata Pomorska-Mól

217 **Novel paramyxovirus causes severe illness in a wildlife biologist working in South Sudan and Uganda**
Cesar G.Albariño, Michael Foltzer, Jonathan Towner, Mike Frace, Shelley Campbell, Carlos M. Jaramillo, Brian Bird, DeeAnn Reeder, Megan Vodzak, Paul Rota, Christina Spiropoulou, Barbara Knust, Stuart T. Nichol, Pierre E. Rollin , Ute Ströher

218 **Type II pneumocytes are the major target cells for seasonal and highly pathogenic influenza A viruses in the human lung**
Viola K. Weinheimer , Anne Becher, Jessica Knepper, Stefan Hippenstiel, Andreas Hocke , Thorsten Wolff

219 **Subacute Sclerosing Panencephalitis: Where does Measles Virus persist inside the host? Some facts and one hypothesis**
Rafael Fernandez-Muñoz1, Juan Carabaña*, Monserrat Caballero*, Miguel A. Muñoz-Alía, and María L. Celma

220 **Fatal encephalitis in African green monkeys and common marmosets after aerosol infection with Rift Valley Fever virus.**
Douglas Reed, Diana Powell, Laura Bethel, Amy Caroline, Anita Trichel, Timothy Oury, and Amy Hartman

221 **Avian Influenza Virus Hemagglutinins H2, H4, H8, and H14 support a highly pathogenic phenotype**
Olga Stech, Jutta Veits, El-Sayed Abdelwhab, Ute Wessels, Siegfried Weber, Angele Breithaupt, Marcus Gräber, Sandra Gohrbandt, Jessica Bogs, Jens P. Teifke, Thomas C. Mettenleiter, Juergen Stech

222 **Establishment and characterization of a highly efficient reverse genetics system for Henipavirus rescue**
Tatyana Yun, Arnold Park, Olivier Pernet, Terry L. Juelich, Jennifer Smith, *Lihong Zhang, Benhur Lee, Alexander N. Freiberg*

223 **Characterization of virulence mechanisms of hemorrhagic fever-causing arenaviruses**
Yuying Liang, Hinh Ly

224 **Immunopathogenesis of Henipavirus infection: role of Interferon type I signalling**
Kevin Dhondt, Cyrille Mathieu, Marie Chalons, and Branka Horvat

225 **Rabies virus-induced expression of chemokines/cytokines enhances the blood-brain-barrier (BBB) permeability in mice**
Qing Q. Chai, Wen Q. H, Zhen F. Fu

226 **A 3D organotypic lung tissue model to study hantavirus pulmonary syndrome**
Karin B. Sundström, Anh Thu Nguyen Hoang, Puran Cheng Shawon Gupta, Clas Ahl, Mattias Svensson and Jonas Klingström

227 **The incubation period is longer than the latent period in chickens and turkeys experimentally infected with Highly Pathogenic Avian influenza viruses**
James Seekings, Chad Fuller, Caroline Warren, June Mynn, Ross Cooper, Elizabeth Aldous, Richard M.

228 **Elements in the Canine Distemper Virus M 3' UTR Contribute to Control of Replication Efficiency and Virulence**
Danielle E. Anderson, Alexandre Castan, Martin Bisaillon, Veronika von Messling

229 **Pathogenicity of H5N1 highly pathogenic avian influenza virus in wild ducks and Herons**
Kosuke Soda, Tatsufumi Usui1, Yukiko Uno, Yasuko Nagai, Hiroichi Ozaki, Hiroshi Ito, Naoki Yamamoto, Tomokazu Tamura, Takahiro Hiono, Masatoshi Okamatsu, Yoshihiro Sakoda, Ayato Takada, Tsuyoshi Yamaguchi, Toshihiro Ito

230 **Interferon type I and T cells determine susceptibility of mice to various Old World Arenaviruses**
Toni Rieger, Doron Merkler , Lisa Oestereich, and Stephan Günther

231 **Ebola virus targets Nrf2-dependent antioxidative stress response via its structural protein VP24.**
St Patrick Reid, Kirill Nemirov, Mathieu Mateo, Audrey Page and Viktor Volchkov.

232 **Length of the NS1 linker region determines pathogenicity of a highly pathogenic H5N1 influenza A virus**
Mirco Schmolke, Benjamin G Hale, Rong Hai1, Jianqiang Ye, Balaji Manicassamy, Daniel R Perez, Adolfo García Sastre

233 **Substitutions T200A and E227A in the hemagglutinin of pandemic 2009 influenza A virus increase lethality but decrease transmissi**
Carles Martínez-Romero, Erik de Vries4), Alan Belicha-Villanueva, Ignacio Mena, Donna M. Tscherne, Virginia L. Gillespie, Randy A. Albrecht, Cornelis A. M. de Haan, Adolfo García-Sastre

234 **Pathogenicity of a pH1N1 influenza virus isolated from a fatal case. Role of host genetics in the outcome of the infection**
Ana Falcon, Ariel Rodríguez, Maite Cuevas, Francisco Pozo, Inmaculada Casas, Juan Ortín, Amelia Nieto

235 **Influenza HA subtypes demonstrate divergent phenotypes for cleavage activation and pH of fusion: implications for host range and**
Summer E. Galloway, Mark L. Reed, Charles J. Russell, David A. Steinhauer

236 **Modeling the Nipah virus transmission cycle**
Emmie de Wit, Joseph B. Prescott, Darryl Falzarano, Trenton Bushmaker, Dana Scott, Vincent J. Munster, Heinz Feldmann

237 **PATHOGENICITY FUNCTIONS OF LYSSAVIRUS GLYCOPROTEINS**
Tobias Nolden, Jens Peter Teifke, Stefan Finke

238 **Analysis of the Immune Response to Sin Nombre Hantavirus in the Rhesus Macaque Model of Hantavirus Cardiopulmonary Syndrome**
Joseph Prescott, David Safronetz, Heinz Feldmann

239 **Role of Neutrophils in the Induction of Pulmonary Edema during Hantavirus Infection in C.B-17Scid Mice**
Jiro Arikawa, Takaaki Koma, Kumiko Yoshimatsu, Noriyo Nagata, Yuko Sato, Kenta Shimizu, Takako Amada, Sanae Nishio, and Hideki Hasegaw

240 **Antigenic and chicken embryonic phenotype differences between Korean-like and G1 lineage H9N2 avian influenza viruses**
Bethany Nash, Alejandro Núñez, Daniel Hicks, Sharon M. Brookes, Ian H. Brown

241 **Genetic Determinants of Junin Virus Attenuation**
Alexey V. Seregin, Nadezhda E. Yun, Slobodan Paessler*X*

242 **Role of different regions of Newcastle disease virus fusion protein for its Pathogenicity**
Sandra Warlich, Christian Grund, Denis Kühnel, Thomas C. Mettenleiter, Angela Römer-Oberdörfer

243 **Molecular diagnostics for Lassa fever at Irrua Specialist Teaching Hospital, Nigeria**
Danny A. Asogun, Donatus I. Adomeh, Jacqueline Ehimuan, Ikponmwonsa Odia, Meike Hass, Martin Gabriel, Stephan Olschlager, Beate Becker-Ziaja, Onikepe Folarin, Eric Phelan, Philomena E. Ehiane, Veritas E. Ifeh, Eghosasere A. Uyigue, Yemisi T. Oladapo, Ekene B. Muoebonam, Osagie Osunde, Andrew Dongo, Peter O. Okokhere, Sylvanus A. Okogbenin, Mojeed Momoh, Sylvester O. Alikah, Odigie C. Akhuemokhan, Peter Imomeh, Maxy A. C. Odike, Stephen Gire, Kristian Andersen, Pardis C. Sabeti, Christian T. Happi, George O. Akpede , Stephan Gunther

244 **Rabies virus phosphoprotein gene functions to facilitate viral neuroinvasion by viral replication in muscle.**
Satoko Yamaoka, Naoto Ito, Keisuke Nakagawa, Kazuma Okada, Kota Okadera, Makoto Sugiyama

245 **Measles virus inclusion bodies may be implicated in development of myopathy**
Hyun-Jeong Kwon, Tomoyuki Honda, Aya Nambu, Tetsuro Arai1, Atsushi Miyakawa, Hiroki Sato, Susumu Nakae, Misako Yoneda, Chieko Kai

246 **Single Mutations in PB2 and NP Mediate Enhanced Pathogenicity of 2009 Pandemic H1N1 Influenza A Viruses in Mice**
Anna Otte, Martina Sauter , Karin Klingel and Gülsah Gabriel

247 **Protection from RVFV neurologic disease is dependent on a functional CD4+ T cell Response**
Kimberly A. Dodd, Anita K. McElroy, Megan E. B. Jones, Stuart T. Nichol, and Christina F. Spiropoulou

248 **Given the high mortality rates and lack of therapeutic treatments associated with the infections they cause, filoviruses are classified as BSL-4 agents.**

Thursday June 20

Session VII: Vaccines and antivirals

Chairpersons :Vaccine part : Beatrice Gomez and Bert Rima

10:00-10:10: Chairpersons's remarks

10:10-10:20 249 **A replication-incompetent influenza virus possessing PspA gene protects mice from influenza virus and S, pneumoniae infection**.
Hiroaki Katsura,ZhenyuPiao, Kiyoko Iwatsuki-Horimoto, Yukihiro Akeda, Kazunori Oishi, Yoshihiro Kawaoka

10:20-10:30	250	**Recombinant influenza virus expressing the F protein of respiratory syncytial virus as a bivalent vaccine** Wendy Fonseca , Makoto Ozawa ,Masato Hatta , Esther Orozco, Máximo B Martínez, Ramón Ocádiz-Ruiz , Yoshihiro Kawaoka.
10:30-10:40	251	**A protective and safe intranasal RSV vaccine based on a recombinant prefusion form of the F protein bound to a bacterium-like pa** Alan Rigter, Ivy Widjaja, HannekeVersantvoort, Maarten van Roosmalen, KeesLeenhouts, Peter J.M. Rottier, **Bert Jan Haijema**, and Cornelis A.M. de Haan,
10:40-10:50	252	**Guiding the immune response against influenza hemagglutinin towards the conserved stalk domain by altering glycosylation** **Dirk Eggink,** Peter Palese
10:50-11:00	253	**Heterosubtypic antiviral activity of influenza virus hemagglutinin-specific antibodies** MiekoMuramatsu, ReikoYoshida, **Ayato Takada**
11:00-11:10	254	**M2e-immunity provides heterosubtypic protection and allows the induction of robust cross-protective T cell responses** Michael Schotsaert, Tine Ysenbaert, KatrijnNeyt, Lorena I. Ibañez, Pieter Bogaert, Bert Schepens, Bart Lambrecht, Walter Fiers&**Xavier Saelens**
11 :10-11 :20	255	**What do we know about the 2009 H1N1 pandemic influenza virus and vaccine?** Z. Chen, C. Cotter, AL Suguitan, X. Cheng, W. Wang, Q. Xu, J. Lu, S. Jacobson, S. Gee, R. Broome and **H. Jin**
11 :20-11 :30	256	**Pseudotyped Newcastle Disease Virus with Paramyxovirus 8 Surface Glycoproteins and Highly Pathogenic Avian Influenza Virus HA** **ConstanzeSteglich**; Christian Grund; Kristina Ramp; Angele Breithaupt; JuttaVeits; Dirk Höper; Günter Keil; Mario Ziller ; HaraldGranzow; Thomas C. Mettenleiter and Angela Römer-Oberdörfer
11:30 -12 :30		Coffee break-Poster session
12:30-12:50	257	**Important immunogenic and antigenic differences between the fusion (F) proteins of hRSV and human metapneumovirus** Laura Rodríguez, Vicente Mas, Olga Cano, Mónica Vázquez, Lorena Ver, M. Carmen Terrón, Daniel Luque, Bernadette G. van den Hoogen, Concepción Palomo and José A. Melero
12:50-13:00	258	**Efficacy of a nonspreading Rift Valley fever virus expressing the Gn glycoprotein from the small genome segment** **Nadia Oreshkova**, RiankaVloet, Rob Moormann, Jeroen Kortekaas
13:00-13:10	259	**Vesicular stomatitis virus-based vaccines protect Syrian hamsters from lethal Nipah virus challenge** **Blair L. DeBuysscher**, Joseph Prescott, Heinz Feldmann

13:10-13:20	260	**Respiratory vaccination with live-attenuated measles virus: studies towards identification of the optimal site of delivery** **Rik de Swart**
13:20-15:00		Lunch- Poster session

Session VII:Vaccines and antivirals continued
Chairpersons:Antiviral part **:**Stephan Becker and Richard Plemper

15:00-15:10 Chair persons's remarks

15:10-15:30	261	**Exploring host targets for broad-spectrum antivirals against respiratory viruses** Daniel J. Chin , CristianCillóniz , Jade Carter , Gong Chen, Grant Beyleveld , Juan Ayllon , Peter Palese , Adolfo García-Sastre, **Megan L. Shaw**
15:30-15:50	262	**MAP kinases and influenza virus infection - Inhibition of p38 MAP kinase protects mice from lethal H5N1 infection** IvonneBoergeling, MircoSchmolke, Katharina Koether, Christina Ehrhardt, **Stephan Ludwig**
15:50-16:00	263	**Hemagglutinin activating host cell proteases provide promising drug targets for the treatment of influenza virus infections** **Eva Böttcher-Friebertshäuser** , Catharina Peitsch , Carolin Tarnow , Daniela Meyer , Torsten Steinmetzer , Hans-Dieter Klenk , Wolfgang Garten
16:00-16:10	264	**A cell-based screening system for influenza A viral RNA transcription/replication inhibitors** **Makoto Ozawa**, Masayuki Shimojima, Hideo Goto, Shinji Watanabe, Yasuko Hatta, Maki Kiso, YousukeFuruta, TaisukeHorimoto, Noel R. Peters, F. Michael Hoffmann, and Yoshihiro Kawaoka
16:10 -17 :10		Coffee break-Poster session
17:10-17:30	265	**Mechanism-Based Covalent Inhibitors of Influenza Virus Neuraminidases show broad spectrum antiviral efficacy in vitro and in vivo** **Jennifer L. McKimm-Breschkin**, Susan Barrett, Victor A. Streltsov, Pat Pilling, Jin-Hyo Kim, Ricardo Resende, Tom Wennekes, Hong-Ming Chen, Nicole Bance, Sabrina Buchini, Andrew G. Watts, Martin Petric5 Richard Liggins, Masahiro Niikura and Stephen G.Withers
17:30-17:40	266	**A cell-based screening system to evaluate the susceptibility of influenza viruses to T-705 (favipiravir)** **Emi Takashita**, Miho Ejima, Seiichiro Fujisaki, Noriko Kishida, Hong Xu, Masaki Imai, Masato Tashiro, Takato Odagiri

17:40-17:50	267	**New generation of fusion inhibitors against paramyxoviruses** Cyrille Mathieu , Samantha G Palmer , Ilaria De Vito , AntonelloPessi , Stefan Niewisk , Branka Horvat , MatteoPorotto and Anne Moscona
20:00		Gala dinner at La Mamounia

Poster Presentation:

268 **Development of a Robust RSV Replicon Assay for High-Throughput Screening**
Choi Lai Tiong-Yip, Helen Plant, Jun Fan, Paul Sharpe, Kirsty Rich, Elise Gorseth, Qin Yu

269 **Non-invasive real-time monitoring of Junín virus infection**
Nadezhda Yun, Alexey Seregin, Milagros Miller and Slobodan Paessler.

270 **Recombinant adenovirus-based DIVA vaccine protects against virulent peste des petits ruminants virus**
Becky Herbert, Jana Baron, Geraldine Taylor, Michael D Baron

271 **A Microparticle Vaccine Containing the CS3C motif of the RSV G Protein Induces Protection Against RSV Infection in Mice**
Patricia Jorquera, Katie Oakley, Thomas J. Powell, and Ralph A. Tripp

272 **Prime-boost vaccination against highly pathogenic avian influenza H5N1 using a Newcastle disease virus vector in day-old chicks**
Helena Lage Ferreira, Fabienne Rauw, Jean François Pirlot, Frédéric Reynard, Thierry van den Berg, Michel Bublot3, Bénédicte Lambrecht

273 **Tyrosine-Kinase Inhibitor (Vandetanib) reduces virus replication and increase survival in a Hantavirus Pulmonary Syndrome model**
Brian H. Bird, Punya Shirvastava-Ranja, Bobbie Rae Erickso, Kimberley A. Dodd, Christina F. Spiropoulou

274 **Screening and optimization of Respiratory Syncytial Virus fusion inhibitors**
Vanessa Gaillard, Julien Héritier, Dominique Garcin, Jean-Manuel Segur, Elodie Baechler, Marc
Mathieu1 and Origène Nyanguile

275 **DNA vaccine provides solid protection in ducks against heterologous H5N1 Avian Influenza Virus challenge**
Junping Li, Shuangcheng Zhao, Guojun Wang, Jinxiong Liu, Pucheng chen, Jianzhong Shi, Xianying Zeng, Yanbing Li, Guohua Deng, Guobin Tian, Yongping Jiang and Hualan Chen

276 **intranasal Vaccination with Integrase-Defective Lentiviral Vectors encoding Influenza Nucleoprotein Induce Protective Immunity t**
J. Fontana, Z. Michelini, D. Negri, A. Cara, M. Salvatore

278 **Development of Recombinant Canine Distemper Virus (rCDV) Vectors for SIV/HIV Vaccine Delivery**
Xinsheng Zhang, Olivia Wallace, Kevin Wright, Palka Sharma, Ninkka Tamot, Mary Lopez, Arban Domi, John Coleman, Gavin Morrow, Christopher Parks

280 **Vaccination with recombinant PIV5 expressing influenza antigens provides diverse immunity and protection against homologous and**
S. Mark Tompkins, Alaina J. Mooney, Zhou Li, Jon D. Gabbard, Dan Dlugolenski, Scott Johnson, Biao He

281 **Virus-like Particles as Vaccines for Paramyxoviruses**
Pramila Walpita

282 **The human respiratory syncytial virus (hRSV) is an ubiquitous pathogen that causes severe lower respiratory tract infection worldwide**
Marie-Anne RAMEIX-WELT, Julien SOURIMANT, Jenna FIX, Aude REMOT, Sabine RIFFAULT, Elyanne GAULT , Jean-Francois ELEOUET

283 **An eight segments swine influenza virus harbouring H1 and H3 hemagglutinins as a LAIV candidate for pigs**
Hyun-Mi Pyo , Aleksandar Masic, Shawn Babiuk and Yan Zhou

284 **Analysis of Complete Genome Sequences of Bovine Parainfluenza Virus Type 3 BN-1 and Vaccine Strain BN-CE for Reverse Genetics**
Takashi Ohkura , Takehiko Kokuho , Misako Konishi , Ken-ichiro Kameyama , Kaoru Takeuchi

285 **2'-Fluoro-2'-deoxypurineriboside ProTides: a step forward towards developing influenza virus polymerase inhibitors**
E. Vanderlinden, S. Meneghesso, A. Brancale, J. Balzarini, C. McGuigan, L. Naesens

286 **NICLOSAMIDE AND CURCUMIN ARE POTENT INHIBITORS OF ARENAVIRUS INFECTION**
Antonella Pasquato, Florian Zoppi, Joel Ramos da Palma, Anggakusuma, Eike Steinmann, and Stefan Kunz *1Institute of Microbiology,*

287 **Generation and evaluation of recombinant, live-attenuated Edmonston-Zagreb measles viruses which express EGFP**
Linda J. Rennick, Rory D. de Vries, Thomas J. Carsillo, Ken Lemon, Geert van Amerongen, Selma Yüksel, R. Joyce Verburgh, W. Paul Duprex1 and Rik L. de Swart

288 **Evaluation of avian paramyxovirus serotype 2-10 as vaccine vectors for chickens pre-immunized against Newcastle disease virus**
Ryota Tsunekuni, Hirokazu Hikono, Takehiko Saito

290 **A novel antiviral compound displays potential broad-spectrum activity through a dual mechanism of action.**
Oliver Dibben, Heinrich Hoffmann, Megan L. Shaw

291 **Two new broadly neutralizing human antibodies binding to the highly conserved stem-epitope of Influenza A hemagglutinin**
Wyrzucki A , Bianchi M (1), Dreyfus C , Blattmann B, Steck M, Kohler I , Grütter M, Wilson I, Hangartner L

292 **Antiviral susceptibilities of A(H3N2)v, A(H1N1)v, A(H1N2)v influenza viruses isolated from humans in the United States in 2011-2**
Katrina Sleeman, Vasiliy P. Mishin, Zhu Guo, Rebecca J. Garten, Amanda Balish, Julie Villanueva, James Stevens, and Larisa V. Gubareva

293 **Rapid strategy for screening by pyrosequencing of influenza reassortants for**
Tatiana Bousse, Svetlana Shcherbik, Marnie Levine and Alexander Klimov

294 **A 900,000 small molecule screen reveals potent anti-influenza and broad-spectrum antiviral compounds.**
Kris M. White, Paul De Jesus, Pablo Abreu, Quy Nguyen, Atsushi Inoue, Silke Stertz, Renate Koenig, Adolfo Garcia-Sastre, Peter Palese, Sumit K. Chanda, and Megan L. Shaw

295 **Antibody-mediated inhibition of Marburg virus budding**
Masahiro Kajihara, Andrea Marzi, Eri Nakayama, Takeshi Noda, Manabu Igarashi, Makoto Kuroda, Rashid Manzoor, Keita Matsuno, Heinz Feldmann, Reiko Yoshida, Yoshihiro Kawaoka, and Ayato Takada

296 **In Vitro and In Vivo Evaluation of DHODH as a Host Target for Antiviral Drug Development**
Nagraj Mani, Joshua Leeman, Raj Kalkeri, Steven Jones, Colleen McNeil, Carlos Faerman, Ioana Davies, Alice Tsai, Mark Ledeboer, Michael Clark, Chao Lin, Rene Rijnbrand, Randy Byrn

297 **In ovo administration of chimeric recombinant Newcastle disease virus expressing VP2 protein of infectious bursal disease virus**
Jinying Ge, Xijun Wang, Meijie Tian, Zhiyuan Wen, Qiulin Feng, Honglei Gao, Xiaole Qi, Xiaomei Wang, and Zhigao Bu

298 **NIAID RESOURCES FOR THE GLOBAL RESEARCH COMMUNITY TO SUPPORT DEVELOPMENT OF NEW VACCINES AND ANTIVIRALS FOR SEVERE VIRAL INFECTI**
Amy E. Krafft, Ph.D.

299 **Real-time monitoring of RSV viral replication in BALB/c mice through detection of luciferase expression using the IVIS® Spectrum**
Joshua DiNapoli, Rachel Groppo, Changhong Zhou, Michael Kishko,

300 **Protective Efficacy of Neutralizing Monoclonal Antibodies in a Nonhuman Primate Model of Ebola Hemorrhagic Fever**
Reiko Yoshida, Andrea Marzi, Yasuhiko Suzuki1, Manabu Igarashi, Friederike Feldmann, Douglas Brining, Heinz Feldmann, and Ayato Takada

301 **A replication-incompetent influenza virus bearing the HN glycoprotein of human parainfluenza virus as a bivalent vaccine**
Hirofumi Kobayashi, Kiyoko Iwatsuki-Horimoto, Maki Kiso, Ryuta Uraki, Yurie Ichiko, Toru Takimoto, Yoshihiro Kawaoka

302 **Live-attenuated respiratory syncytial virus vaccine candidates for clinical studies: Improved genetic stability and multiple mec**
Ursula J. Buchholz , Cindy Luongo, Christine C. Winter, Ruth A. Karron , Roderick S. Tang , Jeanne H. Schickli, and Peter L. Collins

303 **Mutational analysis of the binding pockets of the diketo acid inhibitor L-742,001 in the influenza virus PA endonuclease**
Annelies Stevaert, Roberto Dallocchio, Alessandro Dessì, Nicolino Pala, Dominga Rogolino, Mario Sechi, Lieve Naesens

304 **Investigating the functionality and antigenicity of chimeric lyssavirus glycoproteins and their neutralisation profiles**
Jennifer S Evans, Edward Wright, Andrew J Easton, Anthony R Fooks, Ashley C Banyard

305 **Protection is attributed to both humoral and cellular responses that are reactive against conserved, internal influenza proteins**
Steven F. Bake, Hailong Guo, Randy A. Albrecht, Adolfo García-Sastre, David J. Topham, Luis Martínez-Sobrido

306 **Generation of recombinant arenavirus in FDA-approved cell lines: implications for vaccine development**
Benson Y. H. Cheng, Emilio Ortiz-Riaño, Juan C. de la Torre, Luis Martínez-Sobrido

307 **A PB2-KO influenza virus-based bivalent vaccine protects mice against pandemic H1N1 and highly pathogenic H5N1 virus challenge.**
Ryuta Uraki, Maki Kiso, Kiyoko Iwatsuki-Horimoto, Satoshi Fukuyama, Emi Takashita, Makoto Ozawa, and Yoshihiro Kawaoka

308 **SHe's a novel target for RSV vaccination**
Bert Schepens, Sarah De Baets, Koen Sedeyn, Pieter Bogaert, Brian Gilbert, Pedro A. Piedra, Walter Fiers and Xavier Saelens

309 **FcgammaRIII Is Dispensable for Protection against Influenza by Matrix Protein 2 Ectodomain-Specific IgG2a**
Silvie Van den Hoecke, Karim El Bakkouri, Anouk Smet, Kenny Roose, Michael Schotsaert, Walter Fiers, Xavier Saelens

310 **Hemaggluttinin-Neuriminidase from HPIV3 mediates human NK regulation of T cell proliferation via NKp44 and NKp46.**
Samantha McQuaid, Sinead Loughran, Patrick Power, Dermot Walls, Maria Grazia Cusi, Claes Orvell and Patricia Johnson

	311	**Antiviral compounds that prematurely activate and disable the paramyxovirus fusion Protein** Ilaria De Vito, Laura Palermo, Matteo Porotto and Anne Moscona
	312	**Development of a live attenuated peste des petits ruminants DIVA vaccine using reverse genetics techniques** Murali Muniraju1*, Ashley Banyard2, Hubert Buczkowski , Carrie Batten1 and Satya Parida
	313	**Coordinated viral replication and TLR-3 activation enhances T cell immunity generated by live attenuated influenza vaccines** Vicente Pérez-Giron, Alan Belicha-Villanueva,Sergio Gomez-Medina, Jazmina Gonzalez-Cruz, Anja Lüdtke, Adolfo García-Sastre and César Munoz-Fontela.

Friday June 21

Session VIII: Natural and unnatural virus evolution

Chairpersons :AniceLowen and Stefan Kunz

09:00-09:10:Chair persons's remarks

09:10-09:30	314	**Investigating mechanisms of influenza polymerase host adaptation** Anna Cauldwell, Jason Long, Hongbo Zhou, Olivier Moncorge, and **Wendy Barclay**
09:30-09:40	315	**Evolution of the hemagglutinin of pandemic H1N1 (2009): maintaining optimal receptor binding by compensatory substitutions** **Erik de Vries**, Robert P. de Vries, CarlesMartínez-Romero, Ryan McBride, Peter J.M. Rottier, Adolfo García-Sastre , James C. Paulson, Cornelis A.M. de Haan
09:40-09:50	316	**Hemagglutinin (HA) acid stability regulates H5N1 influenza virus replication, virulence, transmission and interspecies adaptation** Hassan Zaraket, Rebecca M. DuBois, Olga Bridges, Sun-Woo Yoon, Richard J. Webby, Stephen W. White, Robert G. Webster, and **Charles J. Russell**
09:50-10:00	317	**Evolution-guided analysis of primate MxA proteins: the flexible loop L4 is a major determinant for its antiviral specificity** **Corinna Patzina ,** Patrick S. Mitchell , Michael Emerman , Otto Haller , Harmit S. Malik , Georg Kochs
10:00 -11 :00		Coffee break-Poster session
11:00-11:20	318	**Modulating paramyxovirus polymerase function rationally and randomly: towards tunable attenuation** Linda J. Rennick and **W. Paul Duprex**

11:20-11 :30 319 **Evidence for Henipavirus spillover into human populations in Africa.**
Olivier Pernet , Shannon M Beaty , Matthew Lebreton , Brad Schneider, Nathan Wolfe , Benhur Lee

11:30-11 :40 320 **High potential of canine distemper virus in the ability to use macaca and human receptors**
Kouji Sakai, Fumio Seki, Maino Tahara, Noriyuki Otsuki, Yasushi Ami, Ryoji Yamaguchi, Masayuki Saijo, Katsuhiro Komase, Shigeru Morikawa, Makoto Takeda

11:40-11:50 321 **Cell entry pathway of an extinct virus**
Lindsey R. Robinson, MatthijsRaaben, and Sean P.J. Whelan

11 :50-12 :10 322 **Measles virus entry pathway governs efficacy of mantle cell lymphoma radio-virotherapy**
Tanner S. Miest, Marie Frenzke, **Roberto Cattaneo**

12:10 Lunch and beer session

Poster Presentation

323 **New World bats are reservoirs of diverse influenza A viruses**
Suxiang Tong, Xueyong Zhu, Yan Li, Mang Shi, Jing Zhang, Melissa Bourgeois, Hua Yang, Xianfeng Chen, Sergio Recuenco, Jorge Gomez, Li-Mei Chen, Ying Tao1, Cyrille Dreyfus, Wenli Yu, Ryan McBride, Paul J. Carney, Amy T. Gilbert, Jessie Chang, Zhu Guo, Charles T. Davis, James C. Paulson, James Stevens, Charles E. Rupprecht, Edward C. Holmes, Ian A. Wilson, Ruben O. Donis

324 **Sever fever with thrombocytopenia syndrome in Japan**
Masayuki Shimojima1, Toru Takahashi2, Shuetsu Fukushi1, Hideki Tani1, Tomoki Yoshikawa, Tetsuya Mizutani, Shigeru Morikawa, Masayuki Saijo, Ken Maeda

325 **Recent Emergence and Spread of a Phylogenetic Lineage of Rabies Virus in Nepal**
Ganesh R. Pant, Rachel Lavenir, Frank Y. K. Wong, Andrea Certoma, Florence Larrous, Dwij R. Bhatta, Hervé Bourhy, Vittoria Stevens, Laurent Dacheux

326 **Investigations of Marburgvirus Spillover from Natural Populations of Rousettus Aegyptiacus**
Jonathan S. Towner, Brian R. Amman, Serena A. Carroll, Tara K. Sealy, Stuart T. Nichol, Pierre E. Rollin and the Filovirus Bat Ecology Team

327 **Influenza virus reassortment is highly efficient in the absence of segment mismatch**
Nicolle Marshall1, Lalita Priyamvada, Ende, John Steel1, Anice Lowen

328 **Investigating the Dynamics of Filovirus Evolution in Cell Culture**
Kendra Alfson, Anthony Griffiths

329 **Evolution of parainfluenza virus 5**
Bert Rima, Derek Gatherer, Hanna Norsted, Andrew Davison and Richard Randall

330 **Importin-α3 Restricts Influenza Virus Replication in the Mammalian Respiratory Tract**
Swantje Thiele, Debby van Riel, A.D.M.E. Osterhaus, Gülsah Gabriel

331 **Evolutionary analysis of Peste-des-petits ruminants virus**
Pow Murali Muniraju, Muhammad Munir, Ashley Banyard, Jingyue Bao, Aravindh Babu, Mana Mahapatra, Geneviève Libeau, Carrie Batten, Satya Parida

332 **Viral factors affecting the transmission of H1N1 influenza virus**
Patricia J. Campbell, Shamika Danzy, Anice C. Lowen, and John

333 **Genomic analysis of filoviruses associated with 4 hemorrhagic fever outbreaks in Uganda and the Democratic Republic of Congo in**
Cesar Albariño, Trevor Shoemaker, Marina Khristova, Stephen Balinandi, Alex Tumusiime, Shelley Campbell, Deborah Cannon, Aridth Gibbons, Eric Bergeron, Brian Bird, Kimberly Dodd, Christina Spiropoulou, Bobbie Erickson, Lisa Guerrero, Barbara Knust, Stuart Nichol, Pierre Rollin, Ute Ströher

334 **Intrapatient Variation of the Respiratory Syncytial Virus Attachment Protein Gene in patients through time**
Mariana Viegas, Ana Julia Velez Rueda, Alicia S. Mistchenko

335 **Mapping the steps required for the adaptation of animal morbilliviruses to human Cells**
Martin Ludlow, W. Paul Duprex

336 **Characterization of Influenza A(H3N2)v at County and State Agricultural Fairs in the U.S.**
Rebecca Garten, Amanda Balish, Mary Lea Killian, Sabrina Swenson, Andrew Bowman, Charles (Todd) Davis, William Davis, Bo Shu, LaShondra Berman, John Barnes, Stephen Lindstrom, Julie Villanueva, Alexander Klimov

337 **Detection of antibodies to pH1N1 Influenza virus in marine mammals from California in 2009-2012**
Ignacio Mena, Walter M. Boyce, Pamela K. Yochem, Frances M. D. Gulland, Noelia Moreno, Daniel Perez, Ana S. Gonzalez-Reiche, Brent S. Stewart, Adolfo García-Sastre

338 **RNA polymerase II-dependent single infectious RABV cDNAs.**
Alexander Ghanem, Karl-Klaus Conzelmann

339 **Molecular epidemiology of Crimean Congo Hemorrhagic Fever in Iran**
Sadegh Chinikar,Nariman Shah-Hosseini,Sahar Khakifirouz,Tahmineh Jalali,Bahareh Moghassemi,Tahereh Mohammadi,M boubeh Rafigh,Ghazaleh Dadashizadeh

340 **A formula on the number of amino acid substitutions on the hemagglutinin molecules of H3N2 seasonal influenza viruses**
Kimihito Ito, Manabu Igarashi, Ayato Takada

341 **Status of immunity against Seoul virus among wild rats (Rattus norvegicus): implications for persistence of Seoul virus**
Kenta Shimizu, Shumpei P. Yasuda, Kumiko Yoshimatsu, Takaaki Koma, Nguyen Thuy Hoa, Le Thi Quynh Mai, Futoshi Hasebe, Tetsu Yamashiro, Jiro Arikawa

342 **Characterization of the evolutionary relationships among African orthobunyaviruses**
Allison Groseth, Carla Weisend, Eric Dahlstrom, Sarah L. Anzick, Stephen F. Porcella, Hideki Ebihara

344 **A simple method for the detection of tick-borne phleboviruses by one-step RT-PCR - a perfect tool for virus hunting**
Keita Matsuno

345 **Identification of a single point mutation in the 220 loop of equine influenza virus H3 that may play a role in adaptation to dogs**

Adam Rash, Alana Woodward, Liz Medcalf, Emily Holliday, Laura Peachey, Greg Dowd, Janet Daly, John McCauley, Debra Elton.

001. **Fusion activation by parainfluenza virus HN protein**

Sayantan Bose1, Theodore S. Jardetzky3 and Robert A. Lamb1,2,
1Department of Molecular Biosciences and 2Howard Hughes Medical Institute Northwestern University, Evanston, IL 60208-3500 and 3Department of Structural Biology, Stanford University School of Medicine, Stanford, CA 94305

The Paramyxoviridae family of enveloped viruses enters cells through the concerted action of two viral glycoproteins. The receptor-binding protein (HN, H or G) binds its cellular receptor and activates the fusion protein (F), which, through an extensive refolding event, brings viral and cellular membranes together mediating virus-cell fusion. However, the underlying mechanism of F-activation on receptor engagement remains unclear. Current hypotheses propose conformational changes in HN, H or G propagating from the receptor-binding site in the HN, H or G 'globular head' to the F-interacting 'stalk' region. We provide evidence that the receptor-binding globular head domain of the paramyxovirus parainfluenza virus 5 (PIV5) HN protein is entirely dispensable for F-activation. Taking together the crystal structures of HN from different paramyxoviruses, varying energy requirements for fusion activation, F-activation involving the PIV5 HN stalk domain and properties of a chimeric paramyxovirus HN protein, we propose a simple model of paramyxovirus fusion activation.

002. **Unraveling a 3-step Mechanism of Nipah Virus Receptor-Induced Membrane Fusion Triggering**

Qian Liu1, Jacqueline A. Stone1, Birgit Bradel-Tretheway1, Jeffrey Dabundo1, Javier A. Benavides Montano1, Jennifer Santos-Montanez1, Scott B. Biering1, Xiaonan Lu2, Hector C. Aguilar1
1Paul G. Allen School for Global Animal Health, Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, Washington 99164, USA. 2Food, Nutrition, and Health Program, Faculty of Land and Food Systems, University of British Columbia, 2205 East Mall, Vancouver, British Columbia, V6T 1Z4, Canada.

Membrane fusion is essential for entry of the biomedically-important paramyxoviruses into their host cells (viral-cell fusion), and for syncytia formation (cell-cell fusion), often induced by paramyxoviral infections [i.e. those of the deadly Nipah virus (NiV)]. Paramyxovirus membrane fusion requires the coordinated actions of two viral glycoproteins. Upon binding its cell surface receptor, the attachment glycoprotein (HN/H/G) triggers the fusion glycoprotein (F) to undergo conformational changes that merge viral and/or cell membranes. However, there is a knowledge gap on how HN/H/G couples cell receptor binding to F-triggering. Here we report via interdisciplinary approaches the first comprehensive mechanism of NiV membrane fusion triggering, involving three spatiotemporally sequential cell receptor-induced conformational steps. Two conformational changes in two distinct domains of theNiV-G head and one in a NiV-G stalk domain are sequentially required for NiV-F triggering. Moreover, we found that a headless NiV-G mutant is able to trigger NiV-F, and that the NiV-G head's primary fusion modulatory function is to prevent premature triggering of NiV-F on virions by concealing a F-triggering stalk domain until the spatiotemporally correct receptor-binding time and place. Importantly, our results inform our understanding of the general mechanism of paramyxovirus membrane fusion triggering and viral entry.

003 **PVRL4 (Nectin-4) is the epithelial cell for measles and canine distemper viruses and is a target for oncolytic therapy**

Christopher Richardson, Ryan Noyce, Sebastien Delpeut, Ricky Siu
Dalhousie University

Our laboratory recently discovered that PVRL4 (Nectin-4) is the epithelial cell receptor for measles and canine distemper viruses using a comparative microarray analysis [Noyce et al. (2011) PLoS Pathogens 7(8): e1002240; Noyce and Richardson (2012) Trends Micro. 20: 429-439; Noyce et al. (2013) Virology 436: 210-220]. This discovery established a new paradigm for spread of virus from lymphocytes to airway epithelial cells and virus release into the environment. We have investigated the interactions of the V domain of human and canine PVRL4 with the viral H proteins using site-specific mutagenesis and chimeric molecules. Laboratory strains of measles virus use CD46 as a receptor, while vaccine strains of CDV have adapted to use a ubiquitous receptor, which we have also identified. PVRL4 exists at low levels in the adherens junctions of airway epithelial cells, but it is also present in small quantities within the brains of mice, dogs, and humans, accounting for the neurotropic properties of these viruses. Finally,
PVRL4 is a tumor cell marker that is highly expressed on many adenocarcinomas including breast and prostate tumors. Recombinant measles viruses that express green fluorescent protein, luciferase, or the suicide gene, thymidine-active deoxycytidine kinase, were shown to target tumors that express PVRL4. Virus spreads via membrane fusion throughout the tumor, causing cell death. Incorporation of the gene for thymidine-active deoxycytidine kinase into the virus genome, enhances the tumor killing effect of these viruses through use of nucleoside analogues. The oncolytic potential of these viruses has been evaluated in human xenograft/syngeneic mouse tumor models.

004 **The measles virus hemagglutinin β4-β5 hydrophobic groove governs the interactions with nectin-4 and CD46 but not with SLAM**

Mathieu Mateo, Sabriya Syed, Chanakha Navaratnarajah, Roberto Cattaneo
Department of Molecular Medicine, Mayo Clinic, Rochester, Minnesota, USA

To spread in humans, wild type measles virus (MV) relies sequentially on two receptors: the signalling lymphocytic activation molecule SLAM/CD150, expressed on immune cells, and nectin-4/PVRL4, expressed at high levels only in epithelial cells of the upper respiratory tract. Unlike wild type MV, vaccine strains also bind the ubiquitous protein CD46; indiscriminate cell entry through CD46 correlates with attenuation. Co-crystal structures of the MV attachment protein H with its three receptors revealed that all three make contacts with the β4-β5 hydrophobic groove in MV H. However, while nectin-4 and CD46 extend into this groove, SLAM only lays over it. Here we generated multiple H substitution mutants and tested their ability to use each receptor in cell-based fusion assays. These functional assays highlighted a strong overlap between the nectin-4 and CD46 functional binding sites, while the SLAM binding site was easily separated. Moreover, a soluble form of nectin-4 abolished MV entry in CD46-expressing cells but only reduced entry through SLAM. We generated mutant forms of the MV H ectodomain; binding kinetics of selected mutants with the three receptors confirmed a strong overlap of nectin-4 and CD46 binding. These results indicate that the binding modes of the two wild type MV receptors are fundamentally different, while CD46 inhabits the groove used by nectin-4. We are currently attempting to generate a MV retaining entry through CD46 but not nectin-4. This virus will be attenuated but will not be shed, two properties required in the end stage of measles eradication.

005 **Conversion of the parainfluenza virus 5 F protein to a simian virus 41 HN-specific protein by amino acid substitutions**

Masato Tsurudome 1, Morihiro Ito 2, Machiko Nishio 3, Tetsuya Nosaka 1
1 Department of Microbiology and Molecular Genetics, Mie University Graduate School of Medicine, 2-174 Edobashi, Tsu, Mie 514-8507, Japan
2 Department of Biomedical Sciences, College of Life and Health Sciences, Chubu University, 1200 Matsumoto-Cho, Kasugai, Aichi 487-8501, Japan
3 Department of Microbiology, Wakayama Medical University, 811-1 Kimiidera, Wakayama 641-8509, Japan

For most parainfluenza viruses, virus type-specific interaction between the hemagglutinin-neuraminidase (HN) and fusion (F) proteins is prerequisite for mediating virus-cell fusion and cell-cell fusion. The molecular basis of this functional interaction is still obscure partly because it is unknown which region of the F protein is responsible for the physical interaction with the HN protein. Our previous cell-cell fusion assay using the chimeric F proteins of parainfluenza virus 5 (PIV5) and simian virus 41 (SV41) indicated that replacement of two domains in the head region of the PIV5 F protein with the SV41 F counterparts bestowed the PIV5 F protein the ability to induce cell-cell fusion on coexpression with the SV41 HN protein while retaining its ability to induce fusion with the PIV5 HN protein. In the study presented here, we furthered the chimeric analysis of the F proteins of PIV5 and SV41, finding that the PIV5 F protein could be converted to an SV41 HN-specific chimeric F protein by substituting five domains in the head region with the SV41 F counterparts. The SV41 F-derived five domains of this chimera were then divided into 16 segments; nine out of 16 proved to be not involved in determining the specificity for the SV41 HN protein. Finally, mutational analyses of a chimeric F protein, which harbored SV41 F-derived seven segments, revealed that substitution of at most 21 amino acids of the PIV5 F protein with the SV41 F-counterparts was enough to convert the HN protein specificity of the PIV5 F protein.

006 **Vesiculovirus glycoprotein intermediate structures during the fusion associated conformational change.**

Eduard Baquero 1, Aurélie A. Albertini 1, Malika Ouldali 1, Linda Buonocore 2, John K. Rose 2, Jean Lepault 1, Stéphane Bressanelli 1, Yves Gaudin 1

Three different classes of viral fusion proteins have been identified to date based on their common structural motifs. Crystal structures have provided atomic, static pictures of pre- and post-fusion conformations for several of these glycoproteins; however, the transition pathway still remains elusive. VSV G is the prototype of the third class and forms different trimers in both pre- and post-fusion conformations. We report a crystal structure for G of Chandipura virus, another vesiculovirus responsible for deadly encephalopathies. In this single crystal, two distinct conformations corresponding to early and late refolding states of G form a fusion loop-exposing flat heterotetramer with twofold symmetry. Consistent with these data, electron microscopy and tomography show different intermediates at the viral surface depending on experimental conditions. This work reveals the chronological order of the structural changes in the protein and offers a detailed pathway for the conformational transition for the spikes located outside the contact zone with the target membrane. Particularly, our data confirm that the conformational change involves monomeric intermediates and that it likely proceeds to an elongated hairpin monomer before subsequent collapse into the post-fusion trimer. Furthermore, our data and previously published mutagenesis analysis indicate that after dissociation of the pre-fusion trimer into monomers, vesiculovirus fusion glycoprotein could re-associate not only into trimers but also into a dimeric (and even tetrameric) assembly which is eerily similar to that of the pre-fusion shell of the flavivirus fusion glycoprotein

007 **Host cell entry of Respiratory Syncytial Virus involves macropinocytosis followed by proteolytic activation of the F protein**

Magdalena Krzyzaniak, Ari Helenius
Institute of Biochemistry, ETH Zurich, Schafmattstrasse 18, 8093 Zurich, Switzerland

Respiratory Syncytial Virus (RSV) is a highly pathogenic paramyxovirus. We developed assays for RSV endocytosis, intracellular trafficking, membrane fusion, and infection. The results showed that RSV was rapidly and efficiently internalized, and that acid-independent membrane fusion occurred intracellularly after endocytosis. Cell biological studies demonstrated that endocytosis was macropinocytic, and that it was required for infection. The process involved activation of the EGF receptor and its downstream effectors including Cdc42, Pak1, and myosin II. RSV induced transient actin rearrangements accompanied by plasma membrane blebbing, elevated fluid uptake, and internalization of intact RSV particles into large macropinosomes. Expression of a dominant negative Rab5 mutant but not Rab7 decreased infection indicating that RSV penetration is intracellular, and takes place in Rab5 positive macropinosomes before fusion with endolysosomal compartments. The reason why RSV, unlike most paramyxoviruses, depended on endocytic entry was found to be the need for activation of the F protein by a second proteolytic cleavage. It occurred after endocytosis, and involved most likely a furin-like, vacuolar enzyme.

008 **Acid-activated structural reorganization of the Rift Valley fever virus Gc fusion protein**

Matthijn de Boer, Jeroen Kortekaas, Lotte Spel, Peter Rottier, Rob Moormann, Berend Jan Bosch
1 Department of Infectious Diseases and Immunology, Virology Division, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands
2 Central Veterinary Institute of Wageningen University and Research Centre, Lelystad, The Netherlands

Entry of Rift Valley fever virus (RVFV) into the host cell is mediated by the viral glycoproteins Gn and Gc. We investigated the RVFV entry process and, in particular, its pH-dependent activation mechanism using our recently developed RVFV replicon particle system. Entry of the virus into host cells was efficiently inhibited by lysosomotropic agents that prevent endosomal acidification and by compounds that interfere with dynamin- and clathrin-dependent endocytosis. Exposure of plasma membrane-bound virions to an acidic pH (<pH 6) equivalent to the pH of late endolysosomal compartments allowed the virus to bypass the endosomal route of infection. Acid exposure of virions in the absence of target membranes triggered the class II-like Gc fusion protein to form extremely stable oligomers that were resistant to SDS and temperature dissociation and concomitantly compromised virus infectivity. By targeted mutagenesis of conserved histidines in Gn and Gc, we demonstrated that mutation of a single histidine (H857) in Gc completely abrogated virus entry, as well as acid-induced Gc oligomerization. Our data suggest that after endocytic uptake, RVFV traffics to the acidic late endolysosomal compartments, where histidine protonation drives the reorganization of the Gc fusion protein that leads to membrane fusion.

009 **Insights into transport of Marburg virus nucleocapsids in living cells**

Schudt Gordian, Kolesnikova Larissa, Dolnik Olga, Becker Stephan
Institut of Virology, Philipps-University Marburg, Germany

Marburg virus, a filovirus, causes severe hemorrhagic fever in humans and non-human primates with high fatality rates. The viral nucleocapsid is formed by five of the seven structural proteins: The nucleoprotein NP, RNA-dependent polymerase L, polymerase cofactor VP35, VP24, and VP30 and the non-segmented, single stranded, 19.1 kb RNA genome. The nucleocapsid is associated with a regular layer of the matrix protein VP40, which also contacts the viral envelope in which the glycoprotein GP is inserted.
To investigate transport and assembly processes of VP40 and nucleocapsids in living cells VP40 was fused to red fluorescent protein (RFP-VP40) and VP30 was fused to the green fluorescent protein (VP30-GFP). The gene encoding RFP-VP40 was cloned into a plasmid encoding the full-length genome of MARV. Recombinant MARV expressing RFP-VP40 in addition to the authentic VP40 (MARVRFP-VP40) was rescued. To visualize MARVRFP-VP40 infected cells, which transiently expressed nucleocapsid-associated VP30-GFP, dual-color live-cell fluorescence microscopy was established under biosafety level 4 conditions. Using these tools we describe (i) intracellular trafficking of nucleocapsids, (ii) interplay of nucleocapsids with RFP-VP40 and (iii) cytoskeleton components required for transport of nucleocapsids.Key words: Marburg virus - nucleocapsid - VP40 - intracellular trafficking – cytoskeleton

010 **Essential role of the Sendai virus fusion (F) protein in the formation of virus particles**

Manel Essaidi-Laziosi, Anastasia Shevtsova, Carole Bampi, Laurent Roux
Departement of Microbiology and Molecular Medicine University of Geneva Faculty of Medicine Switzerland

Previous studies have shown the importance of thethe fusion protein (F) during Sendai virus particle (VP) production. We are interested in the role of the cytoplasmic domains (CT) of F during this process.The absence of the TYTLE motif of F protein has the same deleterious effect on VP production as the suppression of the entire CT (Fouillot-Coriou, N. and Roux, L. 2000). The mechanism by which TYTLE is involved in this process is not understood. We demonstrate by confocal microscopy and immunoprecipitation that a mutated FTYTLE/5A protein is not expressed at the cell surface although it is still able to interact with the matrix protein (M). Interestingly, the absence of the mutated F at the cell surface correlates with a lower representation of HN and M at cell surface. In addition, these two proteins show a more diffused and disorganized pattern, both at the cell surface and in the cytoplasm. In the end, the mutated F retains M in the cytoplasm and prevents the process of assembly. These results highlight the important role of the fusion protein during virus particle formation.

011 **Orientations of the influenza A viral ribonucleoprotein complexes**

Yukihiko Sugita1, Hiroshi Sagara2, Takeshi Noda1, Yoshihiro Kawaoka1,3,4
1 Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo
2 Medical Proteomics Laboratory, Institute of Medical Science, University of Tokyo
3 Department of Special Pathogens, International Research Center for Infectious Diseases, Institute of Medical Science, University of Tokyo
4 ERATO Infection-induced Host Responses Project, Japan

Background
The influenza A virus possesses an eight-segmented, single-stranded RNA genome (vRNA) of negative polarity. Each vRNA segment binds to multiple copies of viral nucleoproteins together with a small number of heterotrimeric polymerase complexes to form a rod-like ribonucleoprotein complex (RNP). In the RNP, the polymerase complex associates with the conserved 3' and 5' ends, which form the double-stranded vRNA promoter region. Later in infection, eight RNPs are coordinately incorporated into the progeny virion. However, how these RNPs are organized within the virion is not fully understood. Aim To determine the orientation of RNPs within the budding virion.
Methods The fine structure of purified RNPs and their orientations within virions were analyzed by using electron tomography and immuno-electron microscopy.
Results and Discussion
We observed that individual RNPs possess a single polymerase complex at one end of the rod-like structure, and that some RNPs are incorporated into budding virions with their polymerase-binding ends at the budding tip, whereas others align with their polymerase-binding ends at the bottom of the budding virion. These results suggest that interactions among vRNA segments are not restricted to one site within the virion. Our study provides a novel model of influenza virion morphogenesis and important insights

012 **Diverse Morphologies of Influenza Filament Budding: an Ultrastructural Study**

Swetha Vijayakrishnan (1), Colin Loney (1), Dave Jackson (2), David Bhella (1)
(1) MRC-University of Glasgow Centre for Virus Research (CVR), Church Street, Glasgow,UK
(2) Biomedical Sciences Research Complex, University of St Andrews, North Haugh street, St Andrews, UK

Influenza viruses exhibit striking variations in morphology between strains and are characterized as being either filamentous or spherical. This pleomorphic character of the virus poses a huge challenge for structural biology. Clinical isolates have shown to produce long filaments while laboratory-adapted strains are predominantly spherical. However, the function of these virus filaments in the infectious cycle remains undetermined. Here we report the results of the first three-dimensional ultrastructural study of filamentous particles budding from infected cells by cryo-electron tomography (ET) and immunofluorescent confocal microscopy. Filaments often longer than 10 microns were observed, some of which had bulbous heads at the leading end. Although some bulb headed filaments contained tubules that we attribute to M1, none had any recognisable ribonucleoprotein (RNP) genome segments. Long filaments that did not have bulbs were less frequently seen to have an ordered complement of RNPs at their distal ends. ET imaging of purified virus also showed various filament morphologies; short rods (bacilliform) and longer filaments. While bacilliform possessed the classic arrangement of "7+1" RNPs, longer filaments were narrower and mostly lacked this feature but often contained fibrillar material along their entire length. We hypothesise that the important ultrastructural differences between the diverse virus morphologies that we observe suggest distinct assembly pathways and functions. While long filaments containing RNPs may be important for cell-to-cell transmission, empty filaments may serve as a decoy for immune response or help in the spread of smaller virus particles. Thus this work provides novel insights into virus morphology and infection mechanisms.

014 The molecular motor KIF13A mediates intracellular transport of the Lassa virus matrix protein Z

Sarah Katharina Fehling, Wolfgang Garten, Thomas Strecker
Institute of Virology, Philipps-University Marburg, Germany

The matrix protein Z of arenaviruses is the main driving force to promote viral particle production at the plasma membrane. Although multiple functions of Z in the arenaviral life cycle have been uncovered, the mechanism of intracellular transport of Z to the site of virus budding is poorly understood and cellular motor proteins that mediate Z trafficking remained to be identified. We found that the Z protein of the Old World arenavirus Lassa virus (LASV) interacts with the kinesin family member 13A (KIF13A), a plus-end-directed microtubule-dependent motor protein that has been reported previously to transport vesicles containing M6PR from the trans-Golgi-network to the plasma membrane. Overexpression of KIF13A results in relocalization of Z to the cell periphery, while functional blockage of endogenous KIF13A by a dominant-negative mutant or KIF13A-specific siRNA causes a perinuclear accumulation and decreased production of both Z-induced virus-like particles and infectious LASV. The interaction of KIF13A with Z proteins from both Old and New World arenaviruses suggests a conserved intracellular transport mechanism. In contrast, the intracellular distribution of the matrix proteins of prototypic members of the paramyxo- and rhabdovirus family is independent of KIF13A. In summary, our studies identify for the first time a molecular motor protein as a critical mediator for intracellular microtubule-dependent transport of arenavirus matrix proteins.

015 The peste des petits ruminants virus (PPRV) receptors: ovine SLAM and Nectin-4

Jamie Birch 1, Nicholas Juleff 1, Michael P. Heaton 3, Ted Kalbfleisch 4, James Kijas 5 and Dalan Bailey 1,2
1 The Pirbright Institute, Ash Rd., Surrey, UK; 2 The University of Birmingham, Edgbaston, Birmingham, UK; 3U.S. Meat Animal Research Center, Clay Center, Nebraska, USA; 4 School of Medicine, University of Louisville, Louisville, USA; 5 Animal, Food and Health Sciences, CSIRO, Brisbane, Australia.

Peste des petits ruminants virus (PPRV), a Morbillivirus in the Paramyxovirus family, causes a devastating disease of small ruminants (sheep and goats especially) that is prevalent across much of Africa, the Middle East and Southern Asia. Poor subsistence farmers are disproportionately affected by this virus which is capable of causing large outbreaks, with high associated mortality, in naïve hosts. A better understanding of the molecular biology of PPRV is required in order to determine the route of infection, the primary cause of the characteristic viral bronchopneumonia in infected ruminants and the role of host receptor distribution in disease onset and pathogenesis. In addition more sensitive cell lines are required for the isolation and analysis of PPRV. In this study we have demonstrated that ovine Nectin-4 is a permissive receptor for PPRV and mapped the expression of this gene's mRNA (and ovine SLAM, another Morbillivirus receptor) to multiple tissues in the host. Bi- and tri-cistronic lentiviruses encoding combinations of ovine SLAM and/or Nectin-4 were used to generate Morbillivirus-permissive cell lines. In addition we investigated the role of ovine Nectin-4 SNPs in PPRV infection. This lentivirus-based approach was also expanded to probe the interactions between the morbillivirus attachment protein (haemagglutinin) and its viral receptors, SLAM and Nectin-4. We have identified important differences in the interactions between morbilliviruses and these receptors which may determine host-range specificity. Developing a better understanding of the interactions between the virus and its host is a critical step in dissecting the molecular determinants of pathogenicity.

016 **Andes Hantavirus Gn and Gc glycoproteins: Self-assembly into virus-like particles and functions during cell entry**

Rodrigo Acuña, Nicolás Cifuentes, Pierre-Yves Lozach, Nicole Tischler
1-Molecular Virology Laboratory, Fundación Ciencia & Vida, Santiago, Chile.
2-Facultad de Ciencias Biológicas, Universidad Andrés Bello, Santiago, Chile.
3-Laboratory for Cell Biology of Arboviral Infections, INRS-Institut Armand-Frappier, Université du Québec, Québec H7V 1B7, Canada.

Current efforts to understand the basic processes of the replicative cycle of hantaviruses have been in part hampered by the lack of a reverse genetic system and by biosafety requirements. Recombinant Hantaan virus-like particles (VLPs) have been previously described being prepared by co-expression of the viral Gn/Gc glycoproteins and the nucleoprotein. Our current research is focused on understanding the participation of viral proteins in the formation of Andes virus (ANDV) and other hantavirus particles and on the hantavirus cell entry mechanism. ANDV-VLPs were obtained from purified supernatants of transfected cells and characterized by Western blots, electron microscopy and dynamic light scattering. The results show that ANDV Gn and Gc are sufficient to self-assemble into VLPs without the need of other viral components. Further, we investigated the function of Gn and Gc during cell entry. Specifically, we discuss results related with VLP-cell binding, glycoprotein activation and multimerization, membrane interaction and fusion. Funding: FONDECYT 1100756 and CONICYT PFB-16. RA is supported by a CONICYT doctoral fellowship.

017 **Identification of functional domains of class II Rab11-FIPs that participate in the polarized transport of influenza virus vRNP**

Fumitaka Momose, Takashi Ohkura, Yuko Morikawa
Graduate School of Infection Control Sciences, Kitasato University, Tokyo, Japan

Influenza A virus RNA genome is replicated in the nucleus and exists as eight-segmented ribonucleoprotein complexes containing viral RNA polymerase and nucleoprotein (vRNPs). Following the replication, progeny vRNPs are exported to the cytoplasm. We have revealed that cytoplasmic vRNPs are localized to the recycling endosomes, depend on the interaction between GTP-bound Rab11 and viral RNA polymerase, and are finally localized under the apical plasma membrane where packaging of vRNPs and budding of progeny virions occur. We found that the polarized transport of vRNP-positive recycling endosomes was inhibited by co-expression of Rab binding domain (RBD) deletion mutants of class II Rab11-family interacting proteins (Rab11-FIP3/4ΔRBD). These mutants did not inhibit the localization of vRNP to the recycling endosomes but vRNP-positive recycling endosomes were kept in the cytoplasm or were transported to the lateral plasma membrane. Deletion of amino-terminal praline rich region (PRR) did not alter the dominant-negative activity of Rab11-FIP3ΔRBD but further deletion of both PRR and EF-hand domain (EFHD) resulted in the loss of the inhibitory activity. Deletion of carboxyl-terminal Arf binding domain (ABD) also resulted in the loss of the dominant-negative activity. These results suggest that both EFHD and ABD of endogenous class II Rab11-family interacting proteins are required for the apical transport of vRNP-positive recycling endosomes

018 **Inhibition of Membrane Fusion by a mAb interacting with the Morbillivirus Attachment Protein Stalk Domain**

Nadine Ader-Ebert1,2, Andreas Zurbriggen1 and Philippe Plattet1
1Division of Neurological Sciences, DCR-VPH, Vetsuisse faculty, University of Bern, 3001 Bern, Switzerland and 2Graduate School for Cellular and Biomedical Sciences, University of Bern, 3001 Bern, Switzerland.

The MeV and CDV entry systems rely on two interacting envelope glycoproteins: the tetrameric attachment (H) and the trimeric fusion (F) proteins that tightly co-operate to achieve plasma membrane fusion. Each H-monomeric ectodomain consists of a stalk domain supporting a cuboidal head region that contacts different receptors. While the central section of morbillivirus H-stalks "opens" to trigger F, last models suggest that tetrameric H-heads may also move as "dimeric-blocs" to transmit signals for F-activation. We here characterized an anti-CDV-H mAb (1347), which inhibited both viral-cell and cell-cell fusion without influencing receptor-binding. Epitope mapping, using engineered soluble H-forms, revealed a linear segment in the H-stalk domain (126-NPNREFDF-133), which, once transposed into a foreign protein, allowed for its recognition by 1347. MAb-1347 did not cross-react with MeV-H, but substitution of two residues in the epitope by the corresponding CDV-H amino acids (D128N and Y131F) enabled MeV-H detection and cell-cell fusion inhibition. Importantly, the epitope locates membrane-distal from the putative F-contacting segment (residues 110-116), which correlated with 1347-binding activities being unaffected by the presence of F. However, 1347 bound to a membrane-anchored "headless" CDV-H variant (headless-H) with a higher efficiency, suggesting that H-heads, in a pre-receptor-bound conformation, impeded full 1347-recognition. Remarkably, a spontaneous basal level of F-triggering was monitored in headless-H/F-coexpressing cells, and this, regardless of the presence or absence of 1347. Overall, these findings suggest that mAb-1347 does not inhibit F-activation by impairing H-stalks-dependent intrinsic triggering activity. Rather, our preliminary data suggest that mAb-1347 may alter putative receptor-induced H heads-to-stalks "un-clamping" movements.

019 **Interaction between M1 and NP affects influenza virus morphology**

Kristy Bialas, Kendra Bussey, Toru Takimoto
Department of Microbiology and Immunology, University of Rochester Medical Center

Influenza matrix protein 1 (M1) is the key component in viral assembly at the plasma membrane. In addition to initiation of bud formation, M1 mediates efficient genome packaging and is known to be a key determinant of virion morphology. Although the mechanism by which M1 controls virion morphology has not yet been defined, it is suggested that M1 interaction with other viral proteins may play an important role. In this study, we rescued recombinant A/WSN/33 (WSN) virus expressing M1 from A/Aichi/2/68 (Aichi), which differs from WSN by 7 amino acids, and includes those identified to be responsible for filamentous virion formation. Whereas both WSN and Aichi wt viruses produced spherical virions, WSN-AichiM1 exhibited filamentous morphology as detected by immunofluorescence and electron microscopy. Purified WSN-AichiM1 virions also contained less NP compared to wt WSN, consistent with the phenotype of filamentous virions. Additional incorporation of Aichi NP, but not HA, NA, or M2 rescued spherical virion morphology and enhanced levels of NP incorporation, suggesting that specific M1-NP interactions affect virion morphology. Further characterization of viruses containing WSN/Aichi chimeric NPs identified residues 214 and 217 of Aichi NP to necessary and sufficient for the formation of spherical virions by the recombinant WSN-AichiM1 virus. These findings indicate that a specific interaction between M1 and NP or vRNP can determine the morphology of influenza virions.

020 **A Critical Threonine Residue in the Respiratory Syncytial Virus Matrix Protein is required for Matrix Oligomerization and Affect**

Monika Bajorek (1), Sarit Kipper (2), Kim Tran (3), Michael Teng (3), Doron Gerber (2), Reena Ghildyal (4), David Jans (5)
(1)Section of Virology, Faculty of Medicine, Imperial College London, London, UK,(2) Nanotechnology Center, Bar Ilan University, Ramat Gan, Israel,(3)Division of Allergy and Immunology, Department of Internal Medicine, University of South Florida, Tampa, USA,(4)Division of Biomedical Sciences, Faculty of Applied Science, University of Canberra, Canberra, Australia,(5)Department of Biochemistry and Molecular Biology, Monash University, Melbourne, Australia

Human respiratory syncytial virus (RSV), a member of Paramyxovirus family, is the most common cause of bronchiolitis and pneumonia in infants and elderly worldwide. RSV assembles on the apical surface of polarized epithelial cells where viral filaments are formed. These filaments, together with budded spherical virus, are thought to be essential to cell-to-cell spread. We are interested in identifying the key viral and host cell proteins involved in the assembly and budding. The minimal RSV viral proteins essential for filament formation and budding are matrix (M), fusion (F), nucleoprotein (N), and phosphoprotein (P). Here we focus on M protein and its role in filament formation. M proteins are believed to form grid-like arrays on the inner surface of viral membranes while interacting with the glycoproteins and with the nucleocapsids during viral assembly. Recent structural data suggests that Paramyxovirus M proteins must first form dimers that will later assemble into higher order oligomers, probably determining the curvature of plasma membrane in sites of budding. In this study, we identify a key Threonine (Thr) residue that is critical for RSV M assembly. Using biochemical approaches in combination with immunofluorescence staining, we show that RSV M must form dimers in order to be efficiently recruited to viral filaments. Single mutation abolishes M oligomerization. By generating recombinant mutant virus we prove that defects in M assembly results in non-infectious virus production. We suggest that this is partly due to impaired binding to specific cellular proteins on plasma membrane.

021 **A novel bunyavirus causing severe fever with thrombocytopenia syndrome in humans**

Mifang Liang, Guoyu Liu , Jiandong Li, Lina Sun , Chuan Li, Quanfu Zhang, Cong Jin, Xianjun Wang, Shujun Ding, Zheng Xing, Shiwen Wang, Zhenqiang Bi, Dexin Li1.Key *Laboratory for Medical Virology, Ministry of Health, National Institute for Viral Disease Control and Prevention of China CDC, Beijing, China 102206, lidx@chinacdc.cn 2. Shandong Province CDC*

SFTS virus (SFTSV) is a newly identified pathogenic Phlebovirus in the family Bunyaviridae, which causes human severe fever with thrombocytopenia syndrome (SFTS) with an average case fatality rate of 12%. SFTS has been reported in at least 13 provinces in the Central, Eastern, and Northeastern regions of China and also found in USA, Japan and Korea recently. Although the tick Haemophysalis longicornis has been implicated as a vector of SFTSV, the circulation and transmission of SFTSV in nature still remains unclear. To investigate SFTSV infections in ticks and domesticated animals, we sampled a total of 3039 animals and 3141 ticks in two counties of Shandong province, China, from April to November 2011. SFTSV-specific antibodies were detected in 328 of 472 sheep (69.5%), 509 of 842 cattle (60.4%), 136 of 359 dogs (37.9%), 26 of 839 pigs (3.1%), and 250 of 527 chickens (47.4%). SFTSV RNA was detected in all sampled animal species, but the prevalence was low, ranging from 1.7% to 5.3%. Among the collected ticks, the dominant species was H. longicornis ticks, the SFTS viral RNA was detected in about 2% H. longicornis. Phylogenetic analysis of the virus isolates obtained from the sheep, cattle, dog and H. longicornis revealed over 95% homology with SFTSV isolated from patients from the same region, suggesting that the ticks may act as a potential link of SFTSV infections between humans and animals.

022 **Filamentous influenza virases**

Alex Atkins, Matthew Badham, Jeremy Rossman
School of Biosciences, University of Kent

Influenza viruses exist in two distinct morphological forms, filamentous and spherical virions, although the significance of these morphologies is unknown. Recently we have shown that filamentous and spherical virions differ in many basic aspects of viral replication including entry and budding. Whilst many of the interactions required for these processes have been determined, the relevance of the filamentous morphology to viral infection remains poorly defined. Here we analyse a panel of clinical influenza virus isolates and show that morphology is directly related to human infection. Further analysis of the entry and budding of filamentous virions suggests that morphology may affect viral replication, indicating a functional significance of the filamentous morphology.

023 **Respiratory syncytial virus matrix protein: key role in infection**

Reena Ghildyal(1), Michael N. Teng (2), John Mills (3), Philip G. Bardin (4), David A. Jans (5)
(1)Centre for Research in Therapeutic Solutions, Faculty of ESTeM, University of Canberra, Canberra
(2)Department of Internal Medicine, College of Medicine, University of South Florida, Tampa, USA
(3)Department of Medicine, Monash University, Melbourne, Australia
(4)Monash Institute of Medical Research, Monash University, Melbourne, Australia
(5)Department of Biochemistry and Molecular Biology, Monash University, Melbourne, Australia

Respiratory syncytial virus (RSV) is the major respiratory pathogen of infants and major cause of severe respiratory disease in the elderly, causing significant mortality worldwide, with no effective treatment or vaccine available. As in all paramyxoviruses, the RSV matrix protein (M) plays a key role in virus assembly in the host cell cytoplasm. In addition, M has been found to localize in the nucleus of infected cells early in infection, where it acts to inhibit host cell transcription and thereby contribute to pathogenesis. Later in infection, M is central in RSV assembly and budding of RSV, localizing in the cytoplasmic inclusions, co-localising with RSV nucleocapsids and inhibiting its transcriptase activity, perhaps in preparation for packaging and budding, as well as at membranes, co-localising with envelope glycoproteins F and G. We have delineated the various interactions of M with envelope glycoproteins and the nucleocapsid as well as its nuclear trafficking motifs and nuclear import and export pathways. Additionally, we have elucidated its functions in the nucleus and the cytoplasm, establishing the mechanism by which it inhibits host cell transcription, dependent on nuclear localization and nucleic acid binding activity. Our results will enable the development of urgently needed anti-viral agents to tackle RSV infection.

024

Functional characterization of the surface glycoproteins G and F of an African henipa-like bat virus

Nadine Krüger1, Markus Hoffmann1, Jan-Felix Drexler2, Marcel Müller2, Michael Weis3, Andrea Maisner3, Christian Drosten2, Georg Herrler1
Institut für Virologie, Tierärztliche Hochschule Hannover, Hannover, Germany
Institut für Virologie, Universität Bonn, Germany
Institut für Virologie, Philipps-Universität Marburg, Marburg, Germany

The henipaviruses Hendravirus and Nipah virus are among the most pathogenic viruses causing disease in humans. The natural reservoir hosts for henipaviruses appear to be flying foxes of the genus Pteropus. The geographic range of pteropid bats comprises countries of South East Asia and Australia. The existence of African henipa-like viruses was indicated by the presence of neutralizing antibodies in Ghanaian fruit bats and by the identification of genomic RNA in fecal samples from Eidolon helvum. No henipavirus has been isolated so far from African bats. We analysed the functional activity of the F and G proteins of an African henipa-like virus (M74) and found that co-expression in a bat cell line resulted in syncytium formation. Fusion induced by the M74 glycoproteins was less efficient when compared to NiV glycoproteins and characterized by a restricted cell tropism. However, the glycoproteins of M74 and NiV resembled each other in their interaction with the ephrin B2 receptor and in the sensitivity of the fusion activity to cathepsin inhibitors. Our results help to characterize African henipa-like viru es in more detail and provide a basis for isolation of infectious virus from African bats.

025

Molecular basis for small molecule inhibitor of Ebola virus infection that targets receptor NPC1

John Misasi 1 2, Anna Bruchez 1, Marceline Cote 1, Tao Ren 3, Kyungae Lee 3, Soo-mi Lee 1 2, James Cunningham 1
1 Division of Hematology, Department of Medicine, Brigham and Women's Hospital, Boston, MA USA 02115.
2 Division of Infectious Disease, Department of Medicine, Children's Hospital, Boston, MA, USA 02115.
3 New England Regional Center of Excellence for Biodefense and Emerging Infectious Diseases, Harvard Medical School, Boston, MA USA 02115.

Ebola virus (EboV) infection is dependent on proteolytic cleavage of glycoprotein (GP) and binding of cleaved GP1 subunit to receptor Niemann-Pick C1 (NPC1). We identified 3.47, a small molecule inhibitor of infection that targets NPC1 and interferes with binding to cleaved GP1. We analyzed the molecular basis for 3.47 inhibition using resistant viruses and purified proteins. We find that Sudan GP binding and infection are resistant to 3.47 under conditions where Zaire GP is inhibited by >95%. Analysis of chimeric Zaire/Sudan GPs revealed that replacement of residues Ala141-Gln142 in Sudan with Val141-Ser142 from Zaire conferred sensitivity to 3.47 and the reciprocal exchange in Zaire conferred resistance. These exchanges did not alter virus infectivity. Residues 141-2 reside at the apex of a loop that protrudes from the surface of GP1 that makes critical contacts with NPC1. Since 3.47 inhibits GP1 binding to NPC1, we investigated the interaction between NPC1 and 3.47 using purified luminal domains N, C and I from NPC1. We found that luminal domain C specifically binds to cleaved GP1 and is a competitive inhibitor of infection, consistent with published results. We probed the purified luminal domains with 3.98, a 3.47 analog that contains a photoreactive aryl azide, and found that 3.98 forms a covalent adduct with domain C under conditions that inhibit virus infection. Taken together, these findings suggest a model in which the target of 3.47 is a pocket in luminal domain C of NPC1 that must accommodate GP1 Val141-Ser142 for EboV Zaire receptor binding and infection.

026 **Assembly and budding of Rift Valley Fever virus**

Emily A. Bruce1, Robert W. Doms1,2
1Department of Microbiology, University of Pennsylvania, Philadelphia, PA 19104, USA. 2Department of Pathology and Laboratory Medicine, The Children's Hospital of Philadelphia

Rift Valley Fever virus (RVFV) is an enveloped, negative-sense virus that has major economic and public health consequences, causing febrile illness in humans and abortion rates of nearly 100% in pregnant livestock. A member of the Bunyaviridae family, RVFV possesses a tri-partite negative-sense genome, two glycoproteins (GN and G C), a nucleoprotein (N), a polymerase and several non-structural proteins that are dispensable in tissue culture. Little is known about the assembly and budding of RVFV, though the Bunyavirus family is generally thought to bud into membranes derived from the Golgi compartment, before trafficking to the plasma membrane through an undefined route. We confirm that RVFV budding occurs into Golgi cisternae, as observed by transmission electron microscopy. While the glycoproteins rapidly localized to the Golgi compartment after translation, we observed by immunofluorescence and fluorescent in situ hybridization that N and vRNA were localized in a diffuse cytoplasmic pattern at early points in infection and were redirected to Golgi membranes only shortly before budding. Post-budding, we observed virions trafficking to the plasma membrane inside cellular vesicles. In some cases the cellular vesicles appeared elongated and contained multiple virions, suggesting that RVFV may be capable of altering cellular membrane structures during the assembly, budding and final transport phases of its life cycle.

027 **Comparative analysis of matrix ubiquitination and nuclear-cytoplasmic trafficking across the paramyxoviridae genera**

Mickey Pentecost 1, Talia Lester 1, Arnold Park 1, Yao Wang 1, Ajay Vashisht 2, James Wohlschlegel 2, Benhur Lee 1.
1) Department of Microbiology, Immunology and Molecular Genetics, University of California, Los Angeles, CA, USA. 2) Department of Biological Chemistry, University of California, Los Angeles, CA, USA.

The matrix (M) protein of paramyxoviruses is crucial for viral budding from the plasma membrane. We found that the nuclear-cytoplasmic trafficking of Nipah virus M is a prerequisite for budding, and is regulated by a classical bipartite NLS, a leucine-rich NES, and monoubiquitination of the K258 residue within the NLS itself (244RR-X10-RRK258). Interestingly, these motifs are conserved in matrix proteins across *Paramyxoviridae* genera. To study the molecular regulation and evolution of M, we generated NES and the NLS-lysine mutants in matrix proteins from the Henipavirus (Nipah and Hendra), Morbillivirus (Measles), Respirovirus (Sendai), Rubulavirus (Mumps), Avulavirus (Newcastle disease virus), and Pneumovirus (RSV) genera, including the unclassified Tupaia paramyxovirus. Using quantitative 3D confocal microscopy, we show that these motifs control nuclear localization to varying degrees among M proteins and that Nipah-M, Hendra-M and Sendai-M display the most significant and conserved regulation. Notably, intracellular localization of Measles-M was unaffected by mutations in its putative NLS or NES motif. Interestingly, Measles-M and Hendra-M showed significant hetero-oligomerization with Nipah-M, indicated by functional matrix-matrix bimolecular fluorescence complementation (BiFC) and Co-immunoprecipitation, Biochemical studies indicate that nearly all of the representative *Paramyxoviridae* M proteins

028 **Autophagy pathway plays a critical role in entry of Ebolavirus into host cells**

Olena Shtanko and Robert A. Davey
Department of Virology and Immunology, Texas Biomedical Research Institute, San Antonio, TX 78227, USA.

Ebolavirus (EBOV) is a zoonotic, emerging virus which causes severe hemorrhagic fever in humans with up to 90% mortality. Despite much work, there are presently no licensed vaccines or drugs for treatment.
Our and others' previous studies demonstrated that virions enter host cells by macropinocytosis and subsequently traffic through the endocytic network before release of nucleocapsids into the cell cytoplasm. Despite the findings, the mechanism of EBOV entry is complex and poorly understood. Our earlier work showed that the PI3K pathway regulates vesicular trafficking and EBOV entry. While PI3K is known to trigger macropinocytosis, it also associates with Vps34, Beclin-1, p150, and Atg14-like proteins to initiate and coordinate the autophagy pathway, a catabolic process that normally leads to lysosomal degradation of abnormal protein aggregates and organelles. Recently, roles for the autophagy pathway in non-degradative vesicular trafficking have been reported. Here, we demonstrate that disruption of autophagy by siRNA or overexpression of dominant negative forms of the regulatory proteins blocked wild-type EBOV infection. For several viruses, autophagic membranes are important for genome replication, but surprisingly, for EBOV, the autophagy pathway appeared to regulate virus infection in a glycoprotein-dependent manner. Using 3D high-resolution microscopy of virus particle-cell interaction, we show that disrupting formation of autophagic vesicles blocked EBOV at an early entry step. For EBOV, the block appeared novel by preventing virus access to or interaction with a virus receptor or by inhibiting macropinosome internalization. This work was supported by NIH R01AI063513; DTRA HDTRA1-12-1-0002; and the Dou

029 **The impact of GPC and N-terminal region of Lassa virus Z on virus-like particle release**

Shuzo Urata, Jiro Yasuda
Nagasaki University, Institute of Tropical Medicine, JAPAN

Lassa virus (LASV) causes a hemorrhagic fever to human. There is no licensed vaccine against LASV and ribavirin is the only option for treatment, but shows only partial effect and cause significant side effects. Therefore, to understand the life cycle of LASV and develop anti-LASV therapies is an urgent matter.
LASV Z is a viral matrix protein, and its sole expression produces virus-like particle (VLP). L-domain is the short amino acid motif which plays a critical role in virus budding and conserved among many enveloped virus matrix protein. PT/SAP and PPXY are the most characterized L-domains, and interact with Tsg101 and E3 ligase, respectively, to promote VLP production. Two L-domains are located at the C-terminal region of Z, and showed to be important for VLP production. Here, to determine other domain in LASV Z which affects VLP production, we constructed a series of Z protein deletion mutants and examined its VLP production. Most of the mutants did not affect to the VLP production, but only the mutant deleted the amino acid 3-9, Zd3-9, dramatically decreased its VLP production. The GPC expression did not rescue Zd3-9 VLP production, but modified the sensitivity of the VLP production to MbCD treatment in 293T cells. In addition, Z and GPC mediated VLP production was decreased by the treatment with siRNA specific for Tsg101. These data indicate that the N-terminal region of Z is important for the virus budding, and expression of GPC together with Z may reflect the proper LASV budding.

030 **Analysis of Borna disease virus transport**

Caroline Marie Charlier (1), Yuan Ju Wu (2), Sophie Allart (1), Cécile Elisabeth Malnou *(1), Martin Schwemmle (2), Daniel Gonzalez-Dunia (1)*
(1) INSERM U1043, Centre de Physiopathologie de Toulouse Purpan, Paul Sabatier University, Toulouse, France
(2) Department of Virology, University of Freiburg, Freiburg, Germany

Borna disease virus (BDV), a highly neurotropic and non-cytolytic virus, differs from other Mononegavirales by its nuclear site for replication and transcription. The cellular cycle of Bornavirus remains poorly understood, in particular concerning the modalities of intracellular transport of viral ribonucleoparticles (RNP). To track RNP transport in live, infected cells, we constructed a recombinant virus in which a tetracysteine tag was fused to the BDV phosphoprotein (P). Upon rescue and characterization of this recombinant virus, we analyzed viral dynamics in persistently infected cells using live imaging. This analysis revealed that nuclear vSPOTs (viral speckle of transcripts), the viral replication sites, were however very static. We thus performed FRAP (Fluorescence Recovery After Photobleaching) and FLIP (Fluorescence Loss Induced by Photobleaching) experiments, which revealed that one fraction of P protein was very static in vSPOTs, suggesting interaction with cellular partners, whereas another fraction of P was highly mobile and exchanged between all cellular compartments. In coculture experiments, we also observed the early steps of BDV infection by live confocal imaging and visualized viral transfer from cell to cell and its transfer to the nucleus. We also studied the molecular mechanism of BDV transport in primary cultures of neurons. We hypothesized that BDV is transported by specialized vesicles, the axonal endosomes, also used by other viruses such as canine adenovirus. Colocalization studies using immunofluorescence and biochemical purification unambiguously showed that BDV is indeed transported in these Rab7-dependent axonal endosomes. These results open news perspectives for a better characterization of BDV cellular cycle.

031 **The molecular motor KIF13A mediates intracellular transport of the Lassa virus matrix protein Z**

Sarah Katharina Fehling, Wolfgang Garten, Thomas Strecker
Institute of Virology, Philipps-University Marburg, Germany

The matrix protein Z of arenaviruses is the main driving force to promote viral particle production at the plasma membrane. Although multiple functions of Z in the arenaviral life cycle have been uncovered, the mechanism of intracellular transport of Z to the site of virus budding is poorly understood and cellular motor proteins that mediate Z trafficking remained to be identified. We found that the Z protein of the Old World arenavirus Lassa virus (LASV) interacts with the kinesin family member 13A (KIF13A), a plus-end-directed microtubule-dependent motor protein that has been reported previously to transport vesicles containing M6PR from the trans-Golgi-network to the plasma membrane. Overexpression of KIF13A results in relocalization of Z to the cell periphery, while functional blockage of endogenous KIF13A by a dominant-negative mutant or KIF13A-specific siRNA causes a perinuclear accumulation and decreased production of both Z-induced virus-like particles and infectious LASV. The interaction of KIF13A with Z proteins from both Old and New World arenaviruses suggests a conserved intracellular transport mechanism. In contrast, the intracellular distribution of the matrix proteins of prototypic members of the paramyxo- and rhabdovirus family is independent of KIF13A. In summary, our studies identify for the first time a molecular motor protein as a critical mediator for intracellular microtubule-dependent transport of arenavirus matrix proteins.

032 Thermodynamics Tune the Paramyxovirus Membrane Fusion Machinery

Mislay Avila Sánchez 1,2, Lisa Alves 1,2, Mojtaba Khosravi 1,2, Nadine Ader-Ebert 1,2, Andreas Zurbriggen 1 and Philippe Plattet 1
1 Division of Neurological Sciences, DCR-VPH, Vetsuisse Faculty, University of Bern, Switzerland
2 Graduate School for Cellular and Biomedical Sciences, University of Bern, Switzerland.

The Morbillivirus cell entry machinery consists of a fusion (F)-trimer that drastically refolds to mediate membrane fusion following receptor-induced conformational changes in its binding partner, the tetrameric attachment (H) protein. To investigate the molecular determinants that control F refolding, we initially generated F-chimera between measles virus (MeV) and canine distemper virus (CDV) and identified a central pocket within the Morbillivirus F's globular head domain that regulates the stability of the metastable, prefusion conformational state. Most mutants of this "pocket"' appeared to bede-stabilized, a phenotype associated with enhanced membrane fusion activity. Strikingly, under specific triggering conditions, some F-mutants also exhibited resistance to a potent Morbillivirus cell entry inhibitor; a molecule known to enhancethermostabilityof prefusion F-trimersto levels that preclude H-dependent F-triggering. However, in contrast to wild-type F-complexes, we found that de-stabilized F-variants resisted to the antiviral because their low intrinsic thermal stabilities could not be sufficiently raised by the drug.Furthermore, our data revealed that the nature of the triggering combination (receptor-type and H's origin) and the energy level of the prefusion F state act in a concerted manner not only to regulate the initiation of F refolding but also tocontrol resistance to the membrane fusion inhibitor. In conclusion, our results demonstrate that thermodynamics are a key factorcontributing toantiviral drug resistance and to the fineregulation ofthe triggering of the Morbillivirus membrane fusion machinery.

033 Studies on fluorescently labeled Nipah virus matrix protein

Boris Lamp, Erik Dietzel, Marc Ringel, Larissa Kolesnikova, Andrea Maisner
Institute of Virology, Philipps-University Marburg

Nipah virus (NiV) is a zoonotic and highly pathogenic paramyxovirus that causes respiratory or encephalitic diseases in pigs and humans. During *in vivo* infection, epithelial cells of the respiratory and urinary tract become productively infected leading to virus shedding via airway secretions or urine. We recently showed that NiV efficiently spread in epithelia and is predominantly released from the apical surfaces of polarized epithelial cells. While virus spread through the epithelial layer is mediated by glycoprotein-induced cell-to-cell fusion, polarized virus budding is most likely due to the restricted apical sorting of the NiV matrix (M) protein (Lamp, Dietzel et al., 2013).
Aim of this study is to establish tools that allow us to analyze M protein trafficking by live-cell microscopy techniques in NiV-infected polarized epithelial cells. For this purpose, we generated GFP- or mCherry-tagged NiV M protein. Plasmid-encoded M proteins showed an unaltered intracellular distribution, a complete colocalization with untagged M proteins, and an efficient virus-like particle formation. Initial live-cell microscopy studies revealed a rapid accumulation of labeled M proteins at the plasma membrane, often at the tips of filopodia-like protrusions of transfected cells. Ongoing studies in NiV-infected cells address the question if fluorescently labeled M proteins are sufficiently incorporated into infectious particles, and thus allow us in the future to monitor NiV replication in live epithelial cells with recombinant NiV encoding tagged M proteins.

034 **Interaction of Rift Valley Fever virus with the host ESCRT machinery**

Amber Riblett, Emily Bruce, Bob Doms
University of Pennsylvania

Rift Valley Fever virus (RVFV, family Bunyaviridae, genus Phlebovirus) is a negative-sense RNA virus borne by its mosquito vector into livestock and human hosts. Its tripartite genome is packaged into icosahedral virions with four structural proteins: the glycoproteins Gn and Gc, nucleocapsid, and polymerase. In animals, RVFV causes spontaneous abortions; in humans, a febrile illness that is typically self-limiting but can progress to severe symptoms including encephalitis, hepatitis, and hemorrhagic fever. To date, there are no FDA-approved vaccines or therapeutics for this disease, and its viral life cycle within the mammalian host is poorly characterized. We characterize here the interaction of Rift Valley Fever virus with the cell's ESCRT (endosomal sorting complex required for transport) pathway. The ESCRT pathway was first identified ten years ago as a series of protein complexes that act to sort ubiquitinated cargo from endosomes into multivesicular bodies en route to the yeast vacuole (or mammalian lysosome) for degradation. The ESCRT pathway has also been shown to play a role in other cellular processes, among them viral budding, cytokinetic abcission, and autophagy. Viruses that use ESCRT machinery for budding do so by way of late domain motifs contained in cytoplasmically-accessible regions of structural proteins. We examine a putative late domain contained within the cytoplasmic tail of the RVFV Gn glycoprotein, and characterize interactions between RVFV structural proteins and the host ESCRT machinery.

035 **Endosomal Trafficking of the Nipah Virus Fusion Protein**

Michael Weis 1, Lucie Sauerhering 1, Sandra Diederich 2, Andrea Maisner 1
1 Institute of Virology, Philipps-University Marburg, Marburg, Germany
2 Canadian Food Inspection Agency, Winnipeg,Manitoba, Canada

Nipah virus (NiV) is a highly pathogenic, BSL-4 classified paramyxovirus which encodes for two viral surface glycoproteins: the receptor-binding protein G and the fusion protein F. As for all paramyxoviruses, proteolytic activation of the NiV F protein is an indispensable prerequisite for viral infectivity and thus an important pathogenicity factor. In contrast to other ortho- and paramyxoviruses, activation of the NiV F protein takes place within the endosomal-recycling compartment and is mediated by cellular cathepsin L or B. Activation of F therefore depends on a functional YxxΦ endocytosis signal in the cytoplasmic tail of the protein. Aim of the study was to characterize the trafficking pathway of F proteins through the different endosomal-recycling compartments in epithelial cells by colocalization studies with various cellular marker proteins (rab and adaptor proteins). We furthermore generated F truncation mutants to identify NiV F cytoplasmic tail sequences that are involved in correct intracellular transport and which are therefore essential for functional F cleavage and cell-to-cell fusion.

abstracts

036 **Investigating the molecular determinants of mumps virus (MuV) entry and egress**

Connor G. G. Bamford (1), Martin Ludlow (2), Michael R. Wilson (2), Linda J. Rennick (2), Ken Lemon (1), Bertus K. Rima (1), W. Paul Duprex (2)
(1) - Centre for Infection and Immunity, Queen's University, Belfast
(2) - Department of Microbiology, Boston University

Mumps virus (MuV) is a respiratory spread, neurotropic human pathogen. Despite widespread vaccination, large MuV outbreaks continue to occur in the developed world. The mechanisms MuV utilises to infect and cause disease are poorly understood, in part due to lack of well characterized clinical isolates and animal models. We generated a reverse genetics system for the rescue of recombinant (r) MuV based on the sequence of unpassaged, clinical material (MuV/New York/2009 genotype G). This isolate (MuVG09) was obtained from an outbreak with over 4,000 symptomatic cases in many individuals who had been vaccinated twice.
Complete, consensus genome sequences of clinical material and passage 1 isolates were compared. Full-length plasmids were constructed containing the MuVG09 anti-genome and inserted an additional transcription unit encoding enhanced green fluorescent protein (EGFP) between the V/P and matrix (M) genes (position 3). Helper plasmids expressing the nucleocapsid (N), phospho- (P) and large (L) proteins and minigenome constructs expressing EGFP and Gaussia luciferase (Gluc) were used to optimise rescue. rMuVG09 and rMuVG09EGFP(3) were rescued, plaque picked, grown on Vero cells and characterised virologically and molecularly in vitro.
Observation of rMuVG09EGFP(3) infection in vitro highlighted diverse modes of transmission. To determine its mechanistic basis, we generated expression vectors encoding MuVG09 structural proteins: Hemagluttinin-Neuraminidase (HN), Fusion (F) and M and EGFP fused to M. Co-expression of EGFP-M and structural proteins showed that EGFP-M trafficking to assembly sites was HN and F-dependant. The effect on EGFP-M localisation by truncation of the cytoplasmic tail domains of HN and F was investigated.

037 **Temporal analysis of HMPV infection reveals the interplay between viral proteins during the replication cycle**

Farah El Najjar, Brent Hackett and Rebecca E. Dutch
Department of Molecular and Cellular Biochemistry, University of Kentucky

Human metapneumovirus (HMPV) is a novel human respiratory pathogen and a leading cause of respiratory tract infections across all age groups worldwide. HMPV belongs to the Paramyxoviridae family, subfamily Pneumovirinae, which contains enveloped, negative sense, single stranded RNA viruses. The process of forming progeny virus particles requires complex interactions between viral nucleoproteins (N), phosphoproteins (P), matrix (M) proteins and viral glycoproteins. To understand the mechanisms of HMPV replication in more details, we performed a time-course analysis of HMPV infection. Confocal microscopy studies revealed that the subcellular localization of N and P is dramatically altered from punctate, cytoplasmic inclusions at early timepoints to long, filamentous extensions later in infection. HMPV M was cytosolic and did not co-localize with N and P at early timepoints, but at later times in infection M localized with N and P in filaments that form at the plasma membrane and extend between cells. The fusion (F) glycoprotein was also detected in these filamentous extensions. Interestingly, we report that overexpression of P in the absence of infection causes similar morphologies to those seen in late stages of infection. The observed morphology is akin to that of the pneumovirus, human respiratory syncytial virus (HRSV), which forms filaments containing N, P, M and F proteins. Our results are the first to demonstrate the interplay of HMPV proteins throughout the natural course of viral infection. Overall, the data suggest that filament formation may be a distinguishing feature of Pneumovirinae pathogenicity while playing a role in cell-to-cell virus transmission.

038 **A charge-dependent interaction is necessary for Rift Valley fever virus-like particle entry into its host cell**

Maria Baudin, Delowar Hossain, Magnus Evander
Department of Clinical Microbiology, Virology, Umeå University, Umeå, Sweden

Background: Rift Valley fever virus (RVFV) is a mosquito borne Phlebovirus belonging to the species *Bunyaviridae*. It can cause retinitis, encephalitis and haemorrhagic fever. The virus is able to infect animals, humans and mosquitoes but not much is known about the entry mechanisms. It is important to better characterize the determinants for RVFV entry into its host cells.
Methods: The assay was based on RVF virus-like particles (VLPs) containing a reporter gene. Cells or RVF VLPs were treated with various compounds to characterize their effect on RVFV binding to its host cell. The net surface charge of the RVFV glycoproteins was also analysed. Results: Binding of RVF VLPs to cells was dependent on charge and divalent ions. Binding was inhibited by the highly negatively charged heparin. The RVFV Gn glycoprotein had a predicted isoelectric point (pI) of 7.6 and a net positive charge of +1.7 at pH 7.4, suggesting interaction between the Gn ectodomain and the negatively charged cell surface. RVFV Gc on the other hand, was highly negatively charged, -14.2 at pH 7.4, most probably reflecting that Gc is not exposed until after binding. Sialic acid, β3-integrins and heparan sulfate was not important for RVF VLP binding.
Conclusions: The binding of RVFV to host cells was dependent on charge interactions between the cell surface and, most probably, the ectodomain of the RVFV Gn glycoprotein. Divalent ions were also important, while sialic acid, β3-integrins and heparan sulfate had no involvement in RVFV cellular binding.

039 **An upstream open reading frame modulates ebola virus polymerase translation and virus replication**

Reed S. Shabman, Omar Jabado, Christopher F. Basler
Icahn School of Medicine at Mount Sinai

Ebolaviruses possess longer genomes than most other non-segmented negative-strand RNA viruses due in part to long 5’ and 3’ untranslated regions (UTRs). To date, specific functions have not been assigned to these UTRs. With reporter assays, we demonstrated that the Zaire ebolavirus (EBOV) 5’-UTRs lack internal ribosomal entry site function. However, the 5’-UTRs do differentially regulate cap-dependent translation when placed upstream of a GFP reporter gene. Most dramatically, the 5’-UTR derived from the viral polymerase (L) mRNA strongly suppressed translation of GFP. The L 5’-UTR is one of four viral genes to possess upstream AUGs (uAUGs), and ablation of each uAUG enhanced translation of the primary ORF (pORF), most dramatically in the case of the L 5’-UTR. Under conditions where eIF2α was phosphorylated, the presence of the uORF sustained translation of the L pORF, indicating that the uORF modulates L translation in response to cellular stress. To directly address the role of the L uAUG in virus replication, a recombinant EBOV was generated in which the L uAUG was mutated to UCG. Strikingly, mutating two nucleotides outside of previously-defined protein coding and cis-acting regulatory sequences attenuated virus growth to titers 10-100-fold lower than a wild-type virus in Vero and A549 cells. The mutant virus also exhibited decreased viral RNA synthesis as early as 6 hours post-infection and enhanced sensitivity to the stress inducer thapsigargin. Cumulatively, these data identify novel mechanisms by which EBOV regulates its polymerase expression, demonstrate their relevance to virus replication and identify a potential therapeutic target.

040 **Initiation and regulation of respiratory syncytial virus transcription and genome replication**

Laure Deflube, Sarah Noton, Chadene Tremaglio, Rachel Fearns
Department of Microbiology, Boston University Medical School, Boston, MA 02118

We characterized the mechanisms by which the respiratory syncytial virus (RSV) polymerase initiates transcription and RNA replication by reconstituting RNA synthesis *in vitro* using purified polymerase and RNA oligonucleotides, utilizing a minigenome system, and/or analyzing RNA from RSV infected cells. We identified two initiation sites in the leader (Le) and trailer-complement (TrC) promoters, at +1 and +3. Mutations that affected RNA synthesis from the +3 site of the Le also impacted mRNA synthesis from the first and subsequent genes, indicating that the +3 initiation site is responsible for initiating sequential mRNA transcription. Analysis of the +1 and +3 initiation mechanisms using the *in vitro* assay showed that addition of sequence specific primers increased the efficiency of initiation at +1, but not +3, suggesting that two initiation mechanisms are involved. Evidence was also obtained to suggest how the polymerase might be regulated. It was found that substitutions in the capping domain in conserved region V of L inhibited transcription and RNA replication, but at different stages of RNA synthesis, suggesting this region might determine polymerase activity. Furthermore, it was found that in addition to directing RNA synthesis initiation, the TrC promoter sequence could be modified by a back-priming activity, resulting in the addition of nucleotides to the 3´ end of the antigenome RNA. This modification inhibited TrC promoter activity. Together these findings suggest a model to explain how the polymerase initiates transcription and RNA replication and is regulated between mRNA, antigenome and genome synthesis during the viral replication cycle.

041 **Functional insights into the measles virus NTAIL-PXD interaction using site-directed and random mutagenesis**

Antoine Gruet1, Marion Dosnon1, Johnny Habchi1, David Blocquel1, Christophe Bignon1, Yaoling Shu2, Joanna Brunel3, Michael Oglesbee2, Denis Gerlier3 and Sonia Longhi1
1CNRS et Aix-Marseille Université, Architecture et Fonction des Macromolécules Biologiques (AFMB), UMR 7257, Marseille, France
2Dept Veterinary Biosciences, Ohio State University, Columbus, OH, USA.
3CIRI, INSERM U1111, CNRS UMR5308, Université Lyon 1, ENS Lyon, CERVI, Lyon, France.

Recruitment of the measles virus polymerase onto the nucleocapsid template relies on interaction between an alpha-helical Molecular Recognition Element (alpha-MoRE) located within a conserved region (Box2) of the C-terminal intrinsically disordered domain of the nucleoprotein (NTAIL), and the C-terminal X domain (PXD) of the phosphoprotein. Modifying the interaction strength between NTAIL and XD is predicted to modulate the polymerase processivity. The impact of three substitutions in Box2 on PXD-induced NTAIL alpha-helical folding, on NTAIL/PXD affinity and on polymerase rates unveiled rather unexpectedly that a reduced binding strength or a loss of the alpha-helical folding capacity of NTAIL has no obvious impact on the polymerase function. In a fully naive approach, we also submitted the whole NTAIL sequence to random mutagenesis and assessed how amino acid substitutions affect partner recognition using a protein complementation assay based on split-GFP reassembly. NTAIL variants generated by error-prone PCR were picked at random, and characterized in terms of sequence and binding abilities towards PXD. The approach identified determinants of the NTAIL/PXD interaction in good agreement with previous work, but also provided new insights. In particular, we discovered that Box2 is poorly evolvable in terms of binding abilities towards PXD and identified NTAIL regulatory sites that either enhance or dampen the interaction while being located outside the primary interaction site. Selected mutants were introduced into biGene-biSilencing recombinant viruses to assess their impact on transcription and replication to get information on the critical dynamics of the NTAIL/PXD interaction in

abstracts

042 **The EM structure of the complementary cRNP replicative intermediate of influenza A virus and host-pathogen interactions**

Ashley York (1), Juha T. Huiskonen (2), Ervin Fodor (1)
(1) Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, United Kingdom
(2) Oxford Particle Imaging Centre, Division of Structural Biology, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford OX3 7BN, United Kingdom

During influenza A virus infection, genomic single-stranded viral RNA (vRNA) is replicated via a complementary (cRNA) intermediate, both of which are encapsidated in viral nucleoprotein and bound by the viral RNA-dependent RNA polymerase to form viral ribonucleoprotein (vRNP) and complementary viral ribonucleoprotein (cRNP) complexes. The viral polymerase is also responsible for viral transcription by a cap-snatching mechanism, the mRNA products of which are occupied by an ever-changing repertoire of RNA-binding proteins to form dynamic mRNP complexes. Here we report a novel RNA tagging and affinity-purification technique being applied to a negative strand virus for the first time in order to purify ribonucleoproteins from infected cells. Recombinant influenza A viruses have been generated by reverse genetics to contain an RNA tag which binds the *Pseudomonas aeruginosa* phage 7 coat protein with nanomolar affinity, the interaction of which is exploited for the RNA-based affinity purification of influenza A mRNPs, cRNPs and vRNPs from infected cells with high specificity. We use electron microscopy techniques and single-particle analysis to study the elusive structure of the cRNP replicative intermediate. Two-dimensional class averages of the cRNP, derived by simultaneous alignment and classification of over 3,000 particles reveal that the cRNP forms a double helical structure, similar to that of the recently reported vRNP structure. We also use highly sensitive mass spectrometry to identify host cellular factors that interact with purified viral ribonucleoprotein complexes. Taken together, these findings contribute to our understanding of viral replication mechanisms and host-pathogen relationships.

043 **The role of the influenza virus nucleoprotein in viral transcription and replication**

L Turrell (1), EC Hutchinson (1), JW Lyall (2), LS Tiley (2), E Fodor (1) and FT Vreede (1)
(1) Sir William Dunn School of Pathology, University of Oxford, South Parks Road, OxfordOX1 3RE, United Kingdom.
(2) Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge CB3 0ES, United Kingdom.

The eight vRNA genome segments of influenza A virus are bound by the viral RNA polymerase at the 5' and 3' ends and associated with oligomeric nucleoprotein (NP) to form viral ribonucleoprotein (vRNP) complexes. These vRNP complexes carry out both viral transcription and replication within the nucleus of the host cell. The role of NP in viral transcription and replication, although essential, is not well understood. Here we show that NP is not required for transcription and replication of short viral-like RNA templates in vivo, indicating that NP does not determine the mode of initiation or termination by the viral polymerase. We also show that the recruitment of NP to nascent RNP complexes during replication of full-length viral genes is mediated through NP-NP homo-oligomerisation in a 'tail-loop first' orientation. Finally, we have identified a phosphorylation site in the oligomerisation domain of influenza A virus NP and mutating this site causes a significant decrease in both viral transcription and replication. When the equivalent residue in influenza B virus NP is mutated, viral transcription and replication is inhibited, highlighting the importance of this residue in NP function. We propose that phosphorylation of this domain prevents the oligomerisation of NP and that the oligomerisation status of NP within the host cell is controlled by phosphorylation. This work provides new mechanistic insights into the assembly and regulation of viral RNP complexes.

044 **Influenza nucleoprotein N-terminal deletion mutant is deficient in functional vRNP formation and viral RNA expression.**

Abel Sanchez, Christian F. Guerrero-Juarez, Charles C. Peterson, Ryan Laurel, Muriel Makamure, Jose Ramirez, Jacqueline Olivas, and Laura L. Newcomb.
California State University San Bernardino

The influenza RNA dependent RNA polymerase (RdRP) synthesizes viral RNA in the nucleus as functional viral ribonucleoprotein (vRNP) complexes with RNA and nucleoprotein (NP). The N-terminus of NP contains an unconventional nuclear localization signal (NLS) important for initial vRNP nuclear localization and also interacts with host RNA processing factor UAP56. To study the role of the N-terminus of NP aside from its NLS function, we generated an N-terminal NP mutant encoding the conventional NLS from SV40 T-antigen. Using reconstituted vRNP assays we demonstrate this NP mutant is expressed, present in the nucleus, and binds nucleic acids, but is defective for viral RNA expression. The defect is exacerbated with increased vRNA template length; long vRNA templates result in no detectable viral full-length or partial mRNA transcripts, and no NP containing high molecular weight complexes, providing evidence the defect resides in functional vRNP formation and not mRNA elongation. Interestingly, the shortest template, NS vRNA, exhibits only a limited defect. However, we show this is not due to short template length, but rather activity of the NS protein(s). Expression of NS1 rescues the gene expression defect at the protein level, a finding consistent with the role of NS1 as a viral mRNA translational enhancer. NS1 mutant analysis confirms NS1-RNA binding is not required for the rescue and reveals the NS1-CPSF30 interaction surface is essential, a novel finding. Our results support a role for the N-terminus of NP in efficient formation of functional vRNPs and confirm NS proteins have effects on viral gene expression.

abstracts

045 **Phosphorylation of Ebola virus VP30 influences the composition of the viral nucleocapsid complex: Impact on viral transcription**

Nadine Biedenkopf, Stephan Becker
Institute of Virology Philipps-University Marburg Hans-Meerweinstr. 2 35304 Marburg (Germany)

Ebola virus is a non-segmented negative-sense RNA virus causing severe hemorrhagic fever with high fatality rates in humans and nonhuman primates. For transcription of the viral genome four viral proteins are essential: the nucleoprotein NP, the polymerase L, the polymerase cofactor VP35, and VP30. VP30 represents an essential Ebola virus specific transcription factor whose activity is regulated via its phosphorylation state. In contrast to viral transcription, VP30 is not required for viral replication. Using a minigenome assay, we show that phosphorylation of VP30 inhibits viral transcription while viral replication is increased. Furthermore, phosphorylation of VP30 reciprocally regulates a newly described interaction of VP30 with VP35, and strengthens the interaction with NP. Our results indicate a critical role of VP30 phosphorylation for viral transcription and replication, suggesting a mechanism by which VP30 phosphorylation modulates the composition of the viral polymerase complex presumably forming a transcriptase in presence of non-phosphorylated VP30 or a replicase in the presence of phosphorylated VP30.

046 **The mechanism of RNA synthesis inhibition by the matrix protein of vesicular stomatitis virus**

Benjamin Morin1, Philip J. Kranzusch1, Gergely Tekes1, Robin A. Ross2 and Sean P. J. Whelan1

1Department of Microbiology & Immunobiology, Harvard Medical School, 77 Avenue Louis Pasteur, Boston MA 02115.

2NERCE Biomolecule Production Core Facility, 77 Avenue Louis Pasteur, Boston MA 02115.

The RNA synthesis machinery of nonsegmented negative-strand RNA viruses comprises a genomic RNA encased by a nucleocapsid protein (N-RNA), and associated with the RNA dependent RNA polymerase (RdRP). The RdRP resides within a large (L) protein that engages the N-RNA template via a phosphoprotein (P) cofactor which also serves to enhance RdRP processivity. The L-P complex transcribes the genome RNA into a series of discrete monocistronic mRNAs that are capped, methylated and polyadenylated by L. In the presence of ongoing viral protein synthesis the genomic template is replicated through an encapsidated antigenomic intermediate. Assembly of genomic RNA into virions requires the matrix (M)-dependent condensation of the template with a concomitant inhibition of polymerase activity and the acquisition of the glycoprotein studded lipid envelope. Here we define the mechanism by which the polymerase activity is inhibited during the assembly of virions. Using a simple in vitro polymerase assay comprising pure recombinant viral proteins, and synthetic naked RNA templates, we show that M directly inhibits the RdRP activity of L. Inhibition of the RdRP is independent of the presence of P and requires the C-terminal domain of M. We further demonstrate that M inhibits the RdRP by direct binding of L and we measure an affinity constant for the formation of the M-L complex. This work shows that the RdRP activity of L is suppressed by the direct engagement of L by M, a mechanism that ensures that the viral polymerase is packaged into virions.

047 **Recoding the L gene of vesicular stomatitis virus by computer-aided synthesis leads to unexpected biological phenotypes**

Bingyin Wang1 , Gergely Tekes2, Charles Ward1, Steve Skiena1, Bruce Futcher1, Steffen Mueller1, Sean Whelan2, and Eckard Wimmer1.

1Stony Brook University School of Medicine, NY, USA, 2Harvard University School of Medicine, Boston MA, USA

Codon pair bias (CPB), a neglected phenomenon affecting gene expression, results from some synonymous codon pairs disliking or favoring each other. The differences are small but when amplified by recoding and chemical synthesis (producing hundreds of nt changes) robust phenotypes are scored. For example large scale deoptimized (disliking) codons pairs attenuate virulence as shown previously by computation/synthesis of a highly attenuated influenza virus (Flu) suitable as vaccine candidate. A second possibility for genome recoding is scrambling synonymous codons to introduce maximal nt changes by changes of synonymous codon pair ratios. Scrambling does generally not change replication phenotypes unless essential signal(s) are destroyed. Note that our recodings change neither protein sequences nor codon usage. We report unexpected results by scrambling the genome of vesicular stomatitis virus of Mononegavirales, which, in contrast to Flu, expresses genes from a single stranded RNA. We focused on gene L (Lscr), dividing it into segments L1, L2, and L3. VSV containing L1src (858/2574 nt mutations) replicated like VSV in cell culture whereas, astonishingly, VSV L2src (517/1485) and VSV L3src (627/1788) were dead. The latter suggested hitherto unknown replication signals in the L gene that were destroyed by scrambling. Equally surprising was that VSV L1src is highly attenuated in Balb/c mice: whereas wt VSV killed (intra nasally) at LD50 of 10^5 pfu. VSV L1src could hardly kill the animals even at very high dose. However, VSV L1src induced a very robust adaptive immune response on intranasal infection. The reason for this remarkable attn phenotype caused by scrambling L1 is under investigation.

048 **Functional interaction between PB2 and PA polymerase subunits of influenza virus is a critical determinant of the replication of**

Koyu Hara, Yoko Nakazono, Takahito Kashiwagi, Nobuyuki Hamada, Hiroshi Watanabe
Division of Infectious Diseases, Kurume University School of Medicine

The RNA polymerase of influenza A virus plays an important role in virulence and host-range restriction, and also in the regulation of genetic reassortment. Recent studies suggest that the co-incorporation of PA and PB2 from the same H3N2 strain appears to be important for the efficient virus replication; however, the underlying mechanism remains unclear. Here, we reconstituted reassortant ribonucleoprotein (RNP) complexes and demonstrated that the RNP activity was severely impaired when the PA subunit of H3N2 strain A/NT/60/1968 (NT PA) was introduced into H1N1 or H5N1 polymerase. The NT PA did not affect the correct assembly of the polymerase trimeric complex; however, it severely reduced the replication-initiation activity to 47% in vitro, and the accumulation of RNP to 43% in vivo, of that achieved by the H1N1 polymerase, which led to the loss of RNP activity. Mutational analysis demonstrated that PA residues184N and 383N were the major determinants of the inhibitory effect of NT PA and 184N/383N sequences were unique to human H3N2 strains. Significantly, the inhibitory effect of NT PA was specifically relieved by NT PB2, and the PB2 residue K627 played a key role. Our results suggest that PB2 from the same H3N2 strain might be required for overcoming the inhibitory effect of H3N2 PA in the genetic reassortment of influenza virus.

049 **The N-terminal fragment of influenza A virus (H5N1) PB2 subunit strongly inhibits its RNA-dependent RNA polymerase**

Takahito Kashiwagi*, Koyu Hara, Yoko Nakazono, Yusaku Uemura, Yoshihiro Imamura, Nobuyuki Hamada, Hiroshi Watanabe
Department of Infectious Disease, Kurume University School of Medicine, Kurume, Japan

Introduction Influenza A virus has an eight segmented RNA-genome which causes a genetic reassortment to generate newly pandemic influenza viruses. Mathematically, 256 types of influenza viruses can be generated when eight genomes derived from two different influenza strains merge into one viral particle in the host animals. On the other hand, the reassortment of influenza virus is strongly restricted has been shown. Recently we also showed that H5N1 PB2 subunit severely impaired WSN RNP activities, might play an important role for the restriction of the genomic reassortant.

Materials and Methods

In this report, we have extended a previous result that the H5N1 PB2 subunit severely impaired the RNP activity, and applied the PB2 subunit as a competitor against influenza RNA polymerase. To investigate the competitive effect of the PB2 subunit against to the influenza RNP, we have performed RNP reconstitution assay in 293T cell with/without inhibitors, and measured its RNP activity by luciferase reporter assay.

Results and Discussion

N-terminal H5N1 PB2 subunit competitively inhibited the RNP activity. Moreover we have determined the important amino acid on the PB2 subunit for the strongly inhibitory effect. Our results suggested that the N-terminal fragment of H5N1 PB2 subunit becomes a new agent as an inhibitor. We propose that we must have various ways to fight the threat infectious deceases such as influenza virus, and continue this kind of research to accumulate the fundamental data.

050 **Role of non-coding sequences in Arenavirus mRNAs translation**

Sabrina Foscaldi, Alejandra D'Antuono, Nora Lopez
CEVAN-ICT Milstein, CONICET

The genome of Tacaribe virus (TCRV), prototype of the New World arenaviruses, comprises two RNA segments each containing two non-overlapping open reading frames with an ambisense arrangement. TCRV coding sequences are expressed from subgenomic messenger RNAs (mRNAs) transcribed from the 3′ regions of the genomes and antigenomes. Viral mRNAs contain cap structure and short stretches of non-viral nucleotides upstream of viral 5' untranslated regions (UTR). Also, they lack a 3' poly(A) tail, exhibiting a 3' non-coding region predicted to form stable secondary hairpin structures with a suspected role in translation modulation. To analyze the contribution of non-coding sequences to TCRV mRNAs translation, we generated a synthetic transcript mimicking the TCRV Nucleoprotein (N) mRNA, were the N gene was swapped for the one of Firefly Luciferase (LUC). Mutant mRNAs were designed to carry modifications in either the 5' or the 3' non-coding sequence. Translation from these virus-like mRNAs in transfected cells was evidenced by LUC activity. Deletion of the complete 5' UTR caused loss of LUC activity, while significantly reduced levels of reporter gene activity were associated to deletion of the 3' non-coding region. These results suggest that both 5' and 3' non-coding sequences stimulate virus-like mRNA translation. Studies are underway to define the role of viral proteins in this process and to identify structural signatures in viral mRNAs that may be relevant for their translation.

051 **Mutations in the mRNA capping enzyme active site of the VSV L protein induce abnormal transcription initiation and termination**

Tomoaki Ogino, Amiya K. Banerjee
Department of Molecular Genetics, Lerner Research Institute, Cleveland Clinic, Cleveland, OH, USA

mRNA capping is one of essential mRNA processing events that occurs co-transcriptionally. The RNA-dependent RNA polymerase L protein of vesicular stomatitis virus (VSV) forms a covalent enzyme-RNA intermediate for pre-mRNA capping using a nucleophilic histidine residue in the conserved histidine-arginine (HR) motif. To analyze the effects of cap-deficient mutations in the HR motif on mRNA synthesis, we performed in vitro transcription with recombinant L mutants in the presence of the N-RNA template and co-factor P protein. Using this system, we have previously shown that the wild-type L protein efficiently synthesizes full-length N, P, M, and G mRNAs with a cap structure and poly(A) tail. In contrast, cap-deficient L mutants were found to frequently terminate N mRNA synthesis at an early stage of RNA chain elongation, producing 38- and 40-nucleotide pppA-RNAs, although they synthesized the 47-nucleotide leader RNA from the 3 -end of the genomic RNA to similar extents as the wild-type L protein. We also found that these mutants frequently initiate transcription from a cryptic initiation site within the N gene and immediately terminate transcription to yield a 28-nucleotide pppG-RNA. In addition, these mutants produced small amounts of 3 -polyadenylated full-length N mRNA and its shorter form started with 5 -pppG- that is synthesized using another cryptic initiation site. These findings suggest that the HR motif in the L protein and/or pre-mRNA capping is essential to repress aberrant transcription initiation and termination using cryptic signals within the N gene.

052 **A novel regulatory mechanism determining the genome polarity of the Mononegavirales**

Takashi Irie 1, Isao Okamoto 1, Asuka Yoshida 1, Yoshiyuki Nagai 2, Takemasa Sakaguchi 1 *1 Department of Virology, Institute of Biomedical & Health Sciences, Hiroshima University, Japan, 2 Center of Research Network for Infectious Diseases (CRNID), RIKEN, Japan*

The order *Mononegavirales* comprises a huge number of nonsegmented negative-strand RNA viruses (NNSVs). How an NNSV can be an NNSV has been extensively studied using a prototypic species, vesicular stomatitis virus (VSV). It was established that the negative polarity of VSV genome is defined solely by the *cis*-acting replication promoters; the promoter of the replication-intermediate positive-sense antigenome RNA [the sequence complimentary to the 5'-terminal trailer sequence (Tr) of the genome] to direct the negative-sense genome RNA is much more stronger than the promoter, 3'-leader sequence (Le) of the genome to direct the positive sense antigenome RNA, thereby resulting in the preferential production of progeny with the negative-strand RNA genome. The quantitative ratio of genome to antigenome is invariable in cells throughout the lifecycle with great predominance of genome relative to antigenome. In contrast, we show here that a remarkable shift from the early antigenome-dominant to late genome-dominant phase takes place during the single cycle replication of another prototype species, Sendai virus (SeV). This shift appeared to be governed primarily by the expression of the accessory C protein because no such shift occurred and antigenomes were dominant throughout infection with a recombinant SeV with the C gene deleted, generating antigenome-dominant and noninfectious progeny virions. We thus propose for the first time a *trans*-acting regulation for genome polarity decision of an NNSV. A series of Le/Tr swapping studies further suggested the importance of the primary and secondary structures of the promoters in this *trans*-regulation.

053 **Efficient rescue and reverse genetics of viruses from all Paramyxovirinae genera without the use vaccinia-driven T7 polymerase**

Shannon M Beaty 1, Arnold Park 1, Tatyana Yun 2, Olivier Pernet 1, Frederic Vigant 1, Adria Allen 3, Yao Wang 1, Mike Lyons 1, Nancy L. McQueen 4, Alexander N Freiberg 2, Subbiah Elankumaran 3, Paul Duprex 5, Benhur Lee 1
1 University of California, Los Angeles, USA,
2 University of Texas Medical Branch, Galveston, USA,
3 Virginia Polytechnic Institute and State University, USA,
4 Boston University, Massachusetts, USA,
5 California State University at Los Angeles, USA

Reverse genetics systems for rescue of paramyxoviruses are notoriously inefficient. The requirements for high levels of cytoplasmic T7-RNA polymerase (T7-RNAP) to drive initial expression of antigenomic viral transcripts and the N, P and L proteins for jump-starting a productive replication cycle, are inconveniently coupled with the suboptimal codon usage of the T7-bacteriophage gene, and the cytotoxicity of T7-RNAP. Successful rescue generally requires co-infection with T7-RNAP-expressing recombinant vaccinia viruses (rVV). However, increasing restrictions on the use of rVV, and added complications of using a cytotoxic (pox) virus to express a cytotoxic (bacteriophage) gene to rescue a recombinant paramyxovirus is decidedly inefficient, even if successful. We have created a codon-optimized T7 polymerase (T7opt) gene that enables the rescue of recombinant paramyxoviruses entirely from cloned cDNAs using a single-step transfection methodology. This method was used to rescue representative viruses from every Paramyxovirinae genus. The use of T7opt makes efficient rescue of paramyxoviruses possible on highly transfectable cell types (e.g. Vero, and 293T) without rVV-T7-RNAP. Compared to other T7 deliver methods, our T7opt-transfection methodology is faster, safer, more efficient, and allows greater latitude in the ratios of transfected plasmids that result in successful rescue of recombinant viruses. Rescue efficiency was further enhanced, up to 3-logs in some cases, with the addition of a hammerhead ribozyme (Hh-Rbz) sequence in the transcribed antigenome. This Hh-Rbz, engineered between the full T7 promoter and the 3' leader sequence of the recombinant viral genome, ensures adherence to the rule of six by pre-designed autocatalytic cleavage of the transcript-initiating 3Gs.

054 **THE ROLES OF PHOSPHORYLATION OF THE MUMPS VIRUS PHOSPHOPROTEIN IN VIRUS REPLICATION**

Adrian Pickar, Pei Xu, James Zengel, Andrew Elson, Zhuo Li, Zhenhai Chen and Biao He
Department of Infectious Diseases, University of Georgia

Mumps virus (MuV), a paramyxovirus, is a human pathogen that causes an acute infection with symptoms ranging from parotitis to mild meningitis and severe encephalitis. The phosphoprotein (P) and the large protein (L) of MuV form viral RNA-dependent RNA polymerase complex. The P protein is highly phosphorylated. However, the specific role of this phosphorylation is unknown. In this work, we have taken three approaches to investigate the roles of phosphorylation of P in MuV replication. First, we performed a mass spectrometry analysis of P from infected cells and identified T91 and T165 as phosphorylated; however, mutating these residues to alanine resulted in no significant change in a minigenome system. Second, we have taken a bioinformatics approach to identify potential phosphorylation sites. Mutating these putative phosphorylation sites did not affect the functionalities of the P proteins in the mini-genome system. Last, we have systematically mutated all serine and threonine residues of the P protein to alanine and tested these mutants in the minigenome system. We have identified ten S/T residues that were critical for replication and transcription in this system: nine significantly reduced and one significantly enhanced mini-genome activity. We are incorporating these mutations into MuV genome and have recovered some infectious MuV. Using these mutant viruses and mutant proteins, we have investigated the mechanism of viral gene expression regulated by the phosphorylation of P. The work will not only lead to a better understanding of viral RNA transcription and replication

055 **Factors affecting respiratory syncytial virus polymerase activity at the promoters**

Sarah Noton, Laure Deflubé, Chadene Tremaglio, Rachel Fearns
Boston University School of Medicine, Department of Microbiology, Boston, USA

Respiratory syncytial virus (RSV) RNA synthesis was reconstituted *in vitro* using recombinant RSV polymerase and an RNA oligonucleotide template. This assay revealed the RSV polymerase could initiate *de novo* RNA synthesis at positions +1 and +3 of the trailer-complement (TrC) promoter and could add a specific sequence of nucleotides to the 3' terminus of the TrC RNA using a mechanism that involved base pairing of 3'-UG with internal nucleotides 13C and 14A. RNA products consistent with these activities were also detected in RSV infected cells. Analysis of factors that affect these events showed that *de novo* RNA synthesis and 3' template extension were impacted by salt and metal cations. Increasing concentrations of NaCl inhibited all synthesis events, while Mg2+ ions promoted all activities. Addition of Mn2+ as an alternative to Mg2+ supported both 3'-extension and *de novo* initiation, but affected the relative ratios of these products in a concentration dependent manner. Analysis of templates of different lengths showed that nucleotides 1-14 of TrC were sufficient to direct initiation at +1 and +3, but sequence beyond nucleotide 16 was required for 3' template extension. Analysis of the Le promoter showed that while Le RNA was modified by 3'-extension *in vitro*, there was no evidence this occurred during RSV infection. These data suggest that *de novo* RNA synthesis and 3' template extension activities are mediated by different mechanisms, and that in infected cells the efficiency of 3'-extension is controlled by a factor missing from the *in vitro* assay.

056 **An alternative method to determine the 5’ extremities of non-segmented, negative sense RNA viral genomes using positive replicat**

Paul A. Brown, Francois-Xavier Briand, Olivier Guionie, Evelyne Lemaitre, Celine Courtillon, Aurelie Henry, Véronique Jestin and Nicolas Eterradossi *ANSES ploufragan*

Determining the sequence of non-segmented, negative sense RNA viral genomes is far from routine and often requires the application of several techniques. Here, an existing method currently used just for determination of the genomic 3’ extremity was used to determine both the 3’ and 5’ sequence extremities of a newcastle disease virus and an avian metapneumovirus. This was achieved with a single 3’ nucleotide tailing reaction of both the genomic RNA and the full length, positive sense, antigenomic RNA, followed by a single reverse transcription reaction targeted to the common poly nucleotide tails, and then individual PCRs specific for each extremity using PCR primers derived from the sequence of the RT primer or from previously known neighbouring virus sequences. For each virus the method was employed separately. Sequences from both viruses were in agreement with those previously reported for other paramyxoviruses, yet one extra base at the 3’ and one extra base at the 5’ were identified for the avian metapneumovirus studied here. Importantly, the newly determined extremities maintained the complementarity known to exist between the extremities of these viruses. The method was equally successful with both viruses and can be easily tailored to function with other non-segmented, negative sense viruses through minor modification of only the primer sequences.

057 **Establishment of a Nipah virus reverse genetics system**

Erik Dietzel (1), Bevan Sawatsky (2 and 3), Markus Czub (4), Gary Kobinger (5), Veronika von Messling (6), Andrea Maisner (1)
(1) Institute of Virology, Philipps-University Marburg, Marburg, Germany
(2) Department of Microbiology & Immunology and (3) Galveston National Laboratory, University of Texas Medical Branch, Galveston, Texas, U.S.A.
(4) Faculty of Veterinary Medicine, University of Calgary, Calgary, Alberta, Canada
(5) National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada
(6) Veterinary Medicine Division, Paul-Ehrlich-Institute, Langen, Germany

Reverse genetics technology is an indispensable tool to study various aspects of the negative strand RNA virus life cycle. To apply this technology to Nipah virus, a highly pathogenic BSL-4 classified paramyxovirus, we first generated a pBR-based plasmid encoding the full-length antigenome from viral RNA of the NiV Malaysia strain under control of the T7 promoter. A derivative carrying an additional transcription unit expressing the enhanced green fluorescent protein (eGFP) between the glycoprotein and polymerase (L) open reading frames, and helper plasmids encoding the nucleoprotein (N), the phosphoprotein (P) and the L protein genes also under control of the T 7 promoter were produced. After optimization of the relative plasmid concentrations, infection of 293 cells with MVA-T7 and subsequent transfection of the parental or eGFP-carrying full length plasmids together with the helper plasmids led to syncytia formation and production of infectious cell-free virus particles. Using this reverse genetics system, we successfully recovered wildtype and eGFP-expressing recombinant Nipah viruses. Both recombinant viruses displayed similar growth characteristics and reached final titers of 109 TCID50/ml, corresponding to results obtained with the non-recombinant parental strain. This new system will thus allow us to investigate the role of specific mutations and functional domains in the viral context and contribute to our understanding of NiV biology.

058 **Strategies of avian influenza A virus polymerases for efficient replication after zoonotic transmisión**

Benjamin Mänz, Ron Fouchier
Erasmus MC Rótterdam

Influenza A viruses are major human pathogens, for which wild aquatic birds act as the natural host reservoir. Several "species barriers" are known to limit the spread of avian influenza viruses (AIVs) to non-avian hosts. A strong restriction of the AIV polymerase in mammals generally limits the occurrence of beneficial genomic mutations that are needed to overcome these barriers. Several adaptive mutations in the polymerase genes were reported. However, only little information is available about the precise domains, adaptive mutations are located in, and the function of these domains. Moreover, no studies were performed on naturally occurring mutations in the viral promoter structures. The objective of this project was to characterize polymerase domains and promoter mutations involved in host adaptation. Using database information, candidate mutations that evolved in the polymerase and non-coding-regions during natural selection of AIVs in mammals (including humans) were identified. Next these mutations were tested in the context of human cells. We could identify several activity enhancing mutations in the polymerase genes. Modeling these amino acid exchanges into available crystal structures of PB2 and PA revealed a strong clustering, e.g. PB2-740 between the adaptive positions PB2-701 and PB2-627. The intensity of activity increase was dependent on the segment specific non-coding-regions. Currently, we are testing mutations in the viral promoter regions to show how the influenza polymerase and its promoter regulate efficient genome transcription/replication. These data illustrate the flexibility, but simultaneously complexity of the adaptation of the influenza polymerase to a new species.

059 **Defective interfering particles hamper continuous influenza virus propagation**

Timo Frensing 1, Stefan Heldt 1, Ilona Behrendt 1, Ingo Jordan 2, Yvonne Genzel 1, Udo Reichl 1,3
1Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany
2ProBioGen AG, Berlin, Germany
3Chair of Bioprocess Engineering, Otto-von-Guericke-University Magdeburg, Germany

The best protection against the contagious respiratory illness caused by influenza viruses is annual vaccination. However, the current production capacities for influenza vaccines are insufficient to meet increasing global demands. Therefore, we explored the possibility to establish a continuous production process for influenza viruses using the duck-derived suspension cell line AGE1.CR (ProBioGen AG, Germany). To propagate influenza viruses in continuous culture, a two-stage bioreactor setup was used. AGE1.CR cells were cultivated in a first stirred tank bioreactor. Here, an almost constant cell concentration was maintained and cells were constantly fed to a second bioreactor where virus infection and replication took place. Using this two-stage reactor system it was possible to continuously produce influenza viruses. However, subsequent virus titer analysis revealed a periodic increase and decrease of virus titers during the run-time of 17 days. These titer fluctuations were caused by the accumulation of defective interfering particles (DIPs), which were detectable by PCR. With increasing amounts of DIPs the concentration of infectious viruses started to decrease. In turn, total virus titers also declined since DIPs are unable to replicate without complete helper viruses. Subsequently, due to the constant dilution rate, both infectious viruses as well as DIPs were washed out and at a low multiplicity of infection virus replication restored. In the future, this system shall be analyzed in more detail for its suitability to produce influenza viruses and to study aspects of viral evolution including the generation of DIPs.

060 **Effect of nuclear export protein (NEP) of influenza virus on regulation of polymerase activity and viral RNA síntesis**

Hongbo Zhou, Anna V. Cauldwell, Olivier Moncorgé, Wendy S. Barclay
Section of Virology, Department of Medicine, Imperial College London, St. Mary's Campus, London, United Kingdom

The influenza virus nuclear export protein NEP can regulate the synthesis of viral vRNA, cRNA and mRNA and such regulation is independent of its nuclear export function. Currently, the reported effects of NEP on polymerase actvity are not consistent between different research groups who have used different strains of influenza virus in their experiments. What's more, the molecular mechanism of NEP regulation of virus polymerase is unclear. In this study, using human, pig, and avian influenza virus polymerases, firstly, we showed NEP increased the polymerase activity of swine and avian virus but decreased the polymerase activity of human viruses in human 293T cells. Our data indicated a balance between the level of polymerase activity and the regulation of NEP. NEP increased cRNA, mRNA and vRNA synthesis when viral polymerase activity was low, but increase the accumulation of cRNA and decrease the mRNA when viral polymerase activity was higher. The regulation of NEP was not observed on avian cells. Finally we found that using two different NEPs cloned from differnet strains of avian of swine influenza virus showed distinctive effects upon the regulation of polymerase activity. By creating chimeric NEP proteins dervied from these two natural NEPs we defined the key function sites on NEP associated with the regulation of polymerase activity.

061 **Ebola virus: transcriptional RNA editing versus premature poly-adenylation.**

Valentina A. Volchkova, Jaroslav Vorac, Viktor E. Volchkov
CIRI, INSERM U1111, Université Lyon 1, Molecular Basis of Viral Pathogenicity

Ebola virus (EBOV), a member of the Filovirus family causes lethal hemorrhagic fever in man. The EBOV glycoprotein (GP) gene encodes several transcripts due to RNA editing at a conserved site consisting of 7 consecutive uridines. The majority of GP gene transcripts are unedited and encode for secreted sGP, whereas surface GP is synthesized from edited mRNAs containing an extra adenosine inserted at the editing site by viral polymerase. Here we demonstrate for the first time that the editing site can serve as a cryptic transcriptional stop signal causing synthesis of truncated mRNA. Northern blot analysis revealed that nearly 50% of GP mRNAs in EBOV-infected cells represent full-length GP transcripts whereas others are products of premature transcription termination. Sequence analysis of short mRNAs showed a presence of poly (A) tail consisting of 27-44 adenosines and an absence of translation termination codon at the end of reading frame. Expression of this nonstop mRNA was assayed using a truncated poly-adenylated mRNA (tr-mRNA) synthesized in vitro from a plasmid by T7 polymerase. No specific protein was detected when capped tr-mRNA was used for transfection of 293T cells whereas invitro translation of this RNA resulted in synthesis of a 36kDa GP-specific polypeptide. Of note, introduction of stop codon upstream to the poly (A) sequence restored protein synthesis indicating that mammalian cells possess a mechanism preventing expression of aberrant mRNAs. Role and significance of the editing site as well as function of novel GP gene product in replication of EBOV will be discussed.

062 **Development of a reverse genetics system to generate recombinant Marburg virus derived from a bat isolate**

Luke Uebelhoer, Cesar Albarino, Joel Vincent, Stuart Nichol, Jonathan Towner
Centers for Disease Control and Prevention

African marburg- and ebolaviruses, Family Filoviridae, are causative agents of severe viral hemorrhagic fevers. Recent investigations have demonstrated the Egyptian fruit bat (Rousettus aegyptiacus) to be a natural reservoir for marburgviruses. To better understand the viral molecular genetic determinants essential for virus replication in the natural reservoir host, a new reverse genetics system was developed for the reliable rescue of a Marburg virus originally isolated directly from a R. aegyptiacus bat (Bat-371) captured in southwest Uganda. Although virus rescue systems have been previously developed for Marburg (Musoke) and Ebola viruses (Mayinga), such viruses were originally isolated from humans followed by multiple rounds of virus passage in cell culture. These passage histories may have allowed for the accumulation of adaptive mutations detrimental for replication in its reservoir host. To develop a robust system for rescuing Bat-371 Marburg virus, the exact terminal sequences were determined by 5' and 3' RACE, followed by the expression cloning of NP, VP35, VP30 and L ORFs. Using optimized ratios of support plasmids, conditions were developed to efficiently replicate virus mini-genomes, some containing GFP-reporter constructs, under BSL-2 conditions. Plasmids containing full-length genomic sequences were then constructed, including ones containing GFP insertions in various UTRs throughout the genome. Using near-identical optimized conditions, wild type and GFP-containing Bat-371 Marburg viruses were reliably rescued. These recombinant bat-derived Marburg viruses grew to similar high titers in VeroE6 cells and will have utility in controlled laboratory infections of bats from our R. aegyptiacus colony.

063 **Spatiotemporal analysis reveals inclusion bodies as sites of ebolavirus replication**

Thomas Hoenen, Vinod Nair, Reed Shabman, Allison Groseth, Astrid Herwig, Christopher F. Basler, Stephan Becker, Heinz Feldmann
1 Laboratory of Virology, Division of Intramural Research, National Institute of Allergy and InfectiousDiseases, National Institutes of Health, 903 South 4th Street, Hamilton, MT 59840, USA;2 Institut für Virologie, Philipps Universität Marburg, Marburg 35043, Germany; 3 RML Electron Microscopy Unit, Research Technology Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 903 South 4th Street, Hamilton, MT 59840, USA; 4 Department of Microbiology, Mount Sinai School of Medicine, New York 10029, NY, USA

Genome replication and transcription are essential processes in the virus life cycle. For ebolaviruses they have been extensively studied using minigenome systems. A strength of these systems is that they allow dissection of individual aspects of the virus life cycle. To this end, we developed replication-deficient minigenomes, which for the first time allowed replication and transcription to be studied separately, and used them to investigate the regulation of these processes by the ebolavirus proteins. However, since minigenome systems rely on plasmid-driven expression of only a subset of viral components, they likely do not reflect the full complexity of these processes during viral infection. In order to better understand genome replication and transcription in live ebolaviruses, we have generated a number of recombinant viruses expressing reporter genes from additional transcriptional units, or fused to viral genes without significant loss of function of the viral gene products. We then performed spatiotemporal analyses of replication and transcription with these viruses, and could demonstrate that genome transcription begins within two hours post-infection, whereas genome replication is detectable only at much later times. Further, we showed that replication and transcription are localized in inclusion bodies, demonstrating for the first time a function for these virus-induced structures. Interestingly, our data also suggest that several classes of inclusion bodies exist, which we are currently characterizing using correlative light-electron microscopy. These studies demonstrate that combining minigenome systems and recombinant viruses is a powerful approach to study the details of virus genome replication and transcription.

064 **Molecular requirements for inhibiting viral RNA synthesis and host responses by the Nipah virus C protein**

Michael Lo (1), David Karlin (2-3), Stuart Nichol(1), Christina Spiroloulou(1)
1)Centers for Disease Control and Prevention, Viral Special Pathogens Branch, Atlanta, Georgia, USA
2)The Division of Structural Biology, Oxford, United Kingdom
3)Department of Zoology, University of Oxford, United Kingdom

Nipah virus is a highly pathogenic paramyxovirus which has caused fatal human encephalitis in Bangladesh on a near-annual basis since 2001. We have previously demonstrated a role for the Nipah virus (NiV) C protein in inhibiting both viral replication and host antiviral responses. Through the use of protein alignment programs, we identified certain residues/motifs conserved among henipaviruses, as well as others conserved between the henipaviruses and morbilliviruses. In order to determine the functional significance of these conserved residues, we performed targeted mutagenesis these residues within the context of their respective abilities to inhibit Nipah minigenome replication and to inhibit interferon beta (IFN-b) transcription. Our results indicate that residues among several distinct regions of the NiV C protein contribute to protein stability, as well as to its respective abilities to inhibit minigenome replication and IFN-b transcription.

065 **Analysis of MARV Viral Genome Non-coding Regions**

Jesus Alonso, 1,2 Jean L. Patterson, 2
1 - Department of Microbiology, University of Texas Health Science Center San Antonio
2 - Department of Virology and Immunology, Texas Biomedical Research Insitute

The Marburg viruses Musoke (MARV-Mus) and Angola (MARV-Ang) have highly similar genomic sequences. Analysis of viral replication using various assays consistently identified MARV-Ang as the faster replicating virus. Non-coding genomic regions of negative sense RNA viruses are known to play a role in viral gene expression. A comparison of the six non-coding regions using bicistronic minigenomes revealed that the first two non-coding regions (NP / VP35 and VP35 / VP40) differed significantly in their transcriptional regulation. Deletion mutation analysis of the MARV-Mus NP / VP35 region further revealed that the MARV polymerase (L) is able to initiate production of the downstream gene without the presence of highly conserved regulatory signals. Bicistronic minigenome assays also identified the VP30 mRNA 5' untranslated region as an rZAP-targeted RNA motif. Overall, our studies indicate that the high variation of MARV non-coding regions may play a significant role in observed differences in transcription and/or replication.

066 **Elucidating the biochemical determinants for restriction of influenza virus polymerase activity by PB2 627E in mammalian cellswered by TCPDF**

Duncan Paterson, Ervin Fodor
Sir William Dunn School of Pathology, University of Oxford

Most avian influenza viruses do not replicate efficiently in human cells. This is partly due to the low activity of the RNA polymerase of avian influenza viruses in mammalian cells. An E→K adaptive mutation at residue 627 of the PB2 subunit of the polymerase increases the activity of avian-derived virus polymerases in mammalian cells. Accordingly, viral ribonucleoprotein (RNP) reconstitution assays show that a viral polymerase containing PB2 627E characteristic of avian influenza viruses exhibits impaired activity in mammalian cells compared to a viral polymerase that contains PB2 627K characteristic of mammalian-adapted influenza viruses. In contrast, purified viral polymerases containing either PB2 627E or PB2 627K show comparable levels of activity in transcription assays that require no RNP assembly. To reconcile these conflicting observations, we used an NP-independent cell based ranscription/replication assay to assess viral polymerase activity. We found that PB2 627E polymerase inhibition in mammalian cells is independent of NP expression, but is dependent on the length of the viral RNA template. In addition, restriction of PB2 627E polymerase was overcome by mutations specific to the viral RNA template promoter sequence. Consequently, we propose that PB2 627 affects recruitment of the viral RNA promoter by the viral polymerase in mammalian cells.

067 **Phosphorylation of Marburg virus NP and its influence on replication and assembly**

Anne Brueggemann, Olga Dolnik, Stephan Becker
Institute of Virology, Philipps University Marburg, Marburg, Germany

Marburg virus causes severe hemorrhagic fevers among humans and nonhuman primates. The nucleoprotein NP, together with the viral proteins VP30, VP35, VP24 and the polymerase L, form the nucleocapsid that encapsidates the viral RNA. Previous studies have shown that NP is phosphorylated at C-terminal serine and threonine residues. While in infected cells both phosphorylated and unphosphorylated NP can be detected, only the phosphorylated form is incorporated into released viral particles, suggesting a role of the phosphorylation in virogenesis. Seven phosphorylation regions have been identified in NP, some of which have already been studied more in detail.
To study function of phosphorylation of S619 (region VII), two mutants were constructed replacing S619 by aspartate (NP S619D) or alanine (NP S619A) to mimic either a constantly phosphorylated or non-phosphorylated state of the amino acid. Functional investigations of the obtained mutants were performed using an infectious virus-like particle assay as a model system for filoviral infections. Substitution of NP by NP S619A led to increased rates of transcription and/or replication while the phenotype of NP S619D did not significantly differ from wtNP. However, compared to wtNP, mutant NP S619D showed an enhanced incorporation into infectious virus-like particles, suggesting phosphorylation of S619 to regulate the switch from RNA synthesis to assembly.

068 **Involvement of some P residues in the replication complex activity of rabies virus**

Florence Larrous (1, 2), Olivier Delmas (1), Alexander Ghanem (3), Karl Klaus Conzelmann (3), Hervé Bourhy (1)
(1)Institut Pasteur, Unit of Lyssavirus Dynamics and Host Adaptation, 75 724 Paris, France
(2)) Université Paris Diderot, Sorbonne Paris Cité, Cellule Pasteur, rue du docteur Roux, 75015 Paris, France.
(3) Max von Pettenkofer Institute and Gene Center, Ludwig-Maximilians-University Munich, D-81377 Munich, Germany

Lyssaviruses are the causative agents of rabies, which potentially affect all mammals and consistently induced fatal encephalitis in humans. Their genome (single-stranded RNA of negative polarity) encodes five proteins. Among them the nucleoprotein (N), the phosphoprotein (P), and the viral polymerase (L) participate to the viral replication complex. The interaction between these 3 proteins in the replication complex is not well understood due to a lack of high-resolution structural and functional data. Previously our group identified some residues of the P involved in the interaction with N (Assenberg et al. 2010; Delmas et al. 2010). In the present study we focused more specifically on 4 residues of the P: K212, Y213, R260 and Y294 and investigated their role on the replication complex activity. To this aim, a minireplicon system was developed on the basis of a dog rabies virus isolated in Thailand (THA strain). By co-immunoprecipitation and confocal analysis we studied the interaction between the 3 partners of the complex and by Q-PCR we quantified the distribution of the positive and negative sense viral RNA populations produced during the minireplicon assay. Furthermore, a reverse genetics system was developed on the basis of the THA strain to observe the effect of these mutations on the replication of the virus. Some of these mutations allowed the rescue of the THA virus but with a loss of efficiency. We were able to identify that each of the mutants tested act on different steps of the transcription/replication process.

069 **Growth kinetic analysis of recombinant measles viruses containing mutations in the phosphorylation sites of nucleoprotein**

Akihiro Sugai, Hiroki Sato, Misako Yoneda, Chieko Kai
International Research Center for Infectious Diseases and Laboratory Animal Research Center, The Institute of Medical Science, The University of Tokyo

Measles virus nucleoprotein (N protein) is the most abundant viral protein which tightly associates with viral RNA genome to form the N-RNA complex that provides a template for viral RNA synthesis. In our previous study, major phosphorylation sites of N protein have been identified as S479 and S510. However, the functions of these phosphorylation sites have not yet been clarified. In the present study, we rescued recombinant MVs (rMVs) whose phosphorylation sites of N protein were substituted (rMV-S479A, rMV-S510A, and rMV-S479A/S510A) by reverse genetics, and analyses of these recombinants were performed. In the one-step growth experiment, rMVs showed more rapid growth kinetics and N protein accumulation than those of wt-MV, implying that N-phosphorylation had an important role to control the viral growth rate through regulating viral gene expression. On the other hand, the multi-step growth curves revealed that N-phosphorylation intensity correlated inversely with virus yield. Furthermore, excessive N-phosphorylation resulted in the lower stability against RNase and the faster turnover of the viral genome RNA. Taken together, these results suggest that N-phosphorylation is involved in both the viral gene expression and viral genome RNA stability.

070 **Efficient rescue and reverse genetics of viruses from all Paramyxovirinae genera without the use vaccinia-driven T7 polymerase**

Shannon M Beaty1, Arnold Park1, Tatyana Yun2, Olivier Pernet1, Frederic Vigant1, Adria Allen3, Yao Wang1, Michael Lyons1, Linda J. Rennick4, Nancy L. McQueen5, Alexander N Freiberg2, Subbiah Elankumaran3, W. Paul Duprex4, Benhur Lee1
1University of California, Los Angeles, USA, 2University of Texas Medical Branch, Galveston, USA,3Virginia Polytechnic Institute and State University, USA, 4Boston University, Massachusetts, USA,5California State University at Los Angeles

Reverse genetics systems for rescue of paramyxoviruses are notoriously inefficient. The requirements for high levels of cytoplasmic T7-RNA polymerase (T7-RNAP) to drive initial expression of antigenomic viral transcripts and the N, P and L proteins for jump-starting a productive replication cycle, are inconveniently coupled with the sub-optimal codon usage of the T7-bacteriophage gene, and the cytotoxicity of T7-RNAP. Successful rescue generally requires co-infection with T7-RNAP-expressing recombinant vaccinia viruses (rVV). However, increasing restrictions on the use of rVV, and added complications of using a cytotoxic (pox) virus to express a cytotoxic (bacteriophage) gene to rescue a recombinant paramyxovirus is decidedly inefficient, even if successful. We have created a codon-optimized T7 polymerase (T7opt) gene that enables the rescue of recombinant paramyxoviruses entirely from cloned cDNAs using a single-step transfection methodology. This method was used to rescue representative viruses from every Paramyxovirinae genus. The use of T7opt makes efficient rescue of paramyxoviruses possible on highly transfectable cell types (e.g. Vero, and 293T) without rVV-T7-RNAP. Compared to other T7 deliver methods, our T7opt-transfection methodology is faster, safer, more efficient, and allows greater latitude in the ratios of transfected plasmids that result in successful rescue of recombinant viruses. Rescue efficiency was further enhanced, up to 3-logs in some cases, with the addition of a hammerhead ribozyme (Hh-Rbz) sequence in the transcribed antigenome. This Hh-Rbz, engineered between the full T7 promoter and the 3' leader sequence of the recombinant viral genome, ensures adherence to the rule of six by pre-designed autocatalytic cleavage of the transcript-initiating 3Gs.

071 **SELF-ORGANISATION OF THE VESICULAR STOMATITIS VIRUS NUCLEOCAPSID INTO A BULLET SHAPE**

Ambroise Desfosses1,2, Euripedes A. Ribeiro Jr1, Guy Schoehn1,3, Gregory Effantin1, Danielle Blondel4, Delphine Guilligay1, Marc Jamin1, Rob W. H. Ruigrok1 and Irina Gutsche1*

1 Unit of Virus Host Cell Interactions, UJF-EMBL-CNRS UMI 3265, Grenoble, France,
2 Structural and Computational Biology Unit, EMBL Heidelberg, Germany,
3 CEA-CNRS-UJF UMR 5075, Institut de Biologie Structurale-Jean-Pierre Ebel, Grenoble, France,
*4 CNRS UPR 3296, Virologie Moléculaire et Structurale, Gif-sur-Yvette, France * correspondence to: gutsche@embl.fr*

The typical bullet shape of Rhabdoviruses is thought to rely on the matrix protein for stabilising the nucleocapsid coil. In our recent manuscript (Desfosses et al., Nature Communications 2013), we go further in deciphering the mechanism underlying the nucleocapsid folding and demonstrate that information necessary for packing of viral genetic material into helical bullets is actually contained in the nucleoprotein alone. We scrutinize the polymorphism of purified viral and recombinant N-RNA and show that it can fold into bullets in the absence of other viral components solely under the effect of pH and ionic strength. We provide cryoEM reconstructions of both the conical tip and the helical trunk, analyze the conformational variability and/or flexibility of the reconstituted nucleocapsids, in particular in terms of the helical symmetry, and address the issue of constrains imposed by binding of the matrix protein in vitro. On the whole, our findings overturn the current ideas on rhabdovirus assembly and finally brigde the gap between the view of an isolated nucleocapsid template for replication and transcription in its form of an undulating ribbon, commonly accepted since more than 30 years, and the tight bullet-shaped virion skeleton described in the landmark paper of the Hong Zhou group (Ge et al., Science 2010) three years ago. In addition to this breakthrough in the understanding of the bullet assembly, we also provide new insights into the replication process via a very recent 3D EM analysis of yet another flexible helical state of nucleocapsids.

072 **The crystal structure and RNA-binding of an orthomyxovirus nucleoprotein**

Wenjie Zheng 1, John Olson 1, Vikram Vakharia 2, Yizhi Jane Tao 1

1 Department of Biochemistry and Cell Biology, Rice University, Houston, Texas 77005, USA
2 Department of Marine Biotechnology, University of Maryland Baltimore County, Institute of Marine & Environmental Technology, Baltimore, Maryland 21202, USA

Genome packaging for viruses with segmented genomes is often a complex problem. This is particularly true for influenza viruses and other orthomyxoviruses, whose genome consists of multiple negative-sense RNAs encapsidated as ribonucleoprotein (RNP) complexes. To better understand the structural features of orthomyxovirus RNPs that allow them to be packaged, we performed structural/functional studies of the nucleoprotein (NP) of one orthomyxovirus, the infectious salmon anemia virus (ISAV). As the major protein component of the RNPs, ISAV-NP possesses a bi-lobular structure similar to the influenza virus NP. Because both RNA-free and RNA-bound ISAV NP forms stable dimers in solution, we were able to measure the NP RNA binding affinity as well as the stoichiometry using recombinant proteins and synthetic oligos. Our RNA binding analysis revealed that each NP binds ~12 nts of RNA, shorter than the 24-28 nts originally estimated for the influenza A virus NP based on population average. The 12-nt stoichiometry was further confirmed by results from electron microscopy and dynamic light scattering. Our findings thus suggest that NP-free RNA exists on orthomyxovirus RNPs, and selective RNP packaging is likely accomplished through direct RNA-RNA interactions.

073 **Functional and structural studioes on Schmallenberg virus, a newly emerged orthobunyavirus in Europe**

Richard M. Elliott 1,2, Gjon Blakqori 1, Ingeborg C. van Knippenberg 1,2, Elina Koudriakova 1,2 , Ping Li 1,2, Angela McLees 1,2, Xiaohong Shi 1,2, Agnieszka M. Szemiel 1,2, Haohao Dong 1,3 and Changjiang Dong 3
1, Biomedical Sciences Research Complex, School of Biology, University of St Andrews
2, MRC Centre for Virus Research, University of Glasgow
3, Biomedical Research Centre, Norwich Medical School, University of East Anglia

Schmallenberg virus (SBV) is a newly emerged orthobunyavirus that has caused widespread disease in the offspring of cattle, sheep and goats in Europe. Like other orthobunyaviruses the tripartite SBV genome encodes four structural and two nonstructural proteins. The SBV genome segments were cloned as cDNA and a three-plasmid rescue system was established to recover infectious virus. Using this system a recombinant virus lacking expression of the NSs protein, encoded on the S segment, was recovered. The NSs-deleted virus induced interferon in cells, indicating that like other orthobunyaviruses, NSs functions as an interferon antagonist most probably by globally inhibiting host cell metabolism. The SBV nucleoprotein was expressed in bacteria and its structure determined to 3.06Å resolution. The protomer is composed of two domains (N-terminal and C-terminal domains) with flexible N-terminal and C-terminal arms. The N protein has a novel fold, and forms a central positively charged cleft for genomic RNA binding. Structural and functional analyses demonstrate that both N-terminal and C-terminal arms are involved in N-N interaction and oligomerization and play an essential role in viral RNA synthesis. The crystal structure of SBV N in complex with a 42-nucleotide long RNA was also determined, to 2.16 Å resolution. The complex comprises a tetramer of N that encapsidates the RNA as a cross-shape inside the protein ring structure, with each protomer bound to 11 ribonucleotides. The structure suggests that conformational changes of hydrophobic residues in the RNA binding cleft may render the RNA in the RNP accessible to the viral polymerase.

074 **Structural basis for encapsidation of genomic RNA by La Crosse Orthobunyavirus nucleoprotein**

Juan Reguera, Hélène Malet, Friedemann Weber and Stephen Cusack
Juan Reguera, Hélène Malet and Stephen Cusack:
European Molecular Biology Laboratory, and Unit of Virus Host-Cell Interactions (UJF-EMBL-CNRS), France. Friedemann Weber: Institute for Virology, Philipps University Marburg, Germanyred by TCPDF

The nucleoprotein (NP) of segmented negative-strand RNA viruses such as Orthomyxo-, Arena- and Bunyaviruses coats the genomic viral RNA and together with the polymerase forms ribonucleoprotein particles (RNPs) which are both the template for replication and transcription by cap-snatching and are packaged into new virions. We will describe the crystal structure of La Crosse Orthobunyavirus (LACV) NP both RNA free and a tetrameric form with single-stranded RNA bound. LACV NP is a largely helical protein with a fold distinct from other bunyavirus genera nucleoproteins. It binds 11 nucleotides RNA in the positively charged groove between its two lobes and hinged N- and C-terminal arms mediate oligomerization, allowing variable protein-protein interface geometry. Oligomerization and RNA binding are mediated by residues conserved in the Orthobunyavirus genus. In the 2-fold symmetric tetramer, 44 nucleotides bind in a closed ring with sharp bends at the NP-NP interfaces. The RNA is largely inaccessible within a continuous internal groove. Electron microscopy of RNPs released from virions shows them capable of forming a hierarchy of more or less compact irregular helical structures. We discuss how the planar, tetrameric NP-RNA structure might relate to a polar filament that upon supercoiling could be packaged into virions. This work gives insight into the RNA encapsidation and protection function of bunyavirus NP but also highlights the need for dynamic rearrangements of the RNP to give the polymerase access to the template RNA.

075 **Dissecting the Multifunctional Nucleoprotein of Arenaviruses**

Emilio Ortiz-Riaño1, Benson Y.H. Cheng1, Juan C. de la Torre2, Luis Martínez-Sobrido1
1 Department of Microbiology and Immunology, University of Rochester, 601 Elmwood Avenue, Rochester, NY 14642
2 Department of Immunology and Microbial Science, The Scripps Research Institute, La Jolla, California 92037

Arenaviruses are enveloped viruses with a negative-sense bi-segmented RNA genome. Several arenaviruses cause severe hemorrhagic fever (HF) in humans and pose important public health problems in their endemic regions. These concerns are aggravated by the potential for person-to-person transmission, the lack of Food and Drug Administration-licensed vaccines and current therapeutic intervention limited to an off-label use of ribavirin. Arenavirus genome encodes only four proteins: the RNA-dependent RNA-polymerase (L), the matrix-like protein (Z), the glycoprotein (GP), and the nucleoprotein (NP). NP encapsidates the viral genome, and together with L, is involved in viral replication and transcription. Additionally, NP counteracts the type I interferon (IFN-I) response, an activity mediated by specific amino acid residues located within its C-terminal region. There is however very limited information about the interactions of NP with other viral components. We have characterized domains in the NP of the prototypic arenavirus Lymphocytic Choriomeningitis Virus (LCMV) that are involved in self-association (NP-NP) and interaction with Z (NP-Z). We mapped the domains involved in NP-NP interaction within the N-terminal region, while the NP-Z interaction was mapped to the C-terminal region of NP, overlapping with the previously described anti-IFN-I domain. However, specific amino acid mutations affecting the NP anti-IFN-I function did not affect its interaction with Z, indicating that different residues contribute to these two distinct NP functions. A detailed characterization of NP domains required for its interaction with other viral, as well as cellular, proteins might uncover novel targets for the development of antiviral drugs to combat human pathogenic arenaviruses.

076 **Structural analyses of influenza A virus polymerase and RNPs**

Rocío Arranz2, Rocío Coloma1, Patricia Resa-Infante1, Noelia Zamarreño1, Maria Angeles Recuero-Checa3, Roberto Melero3, José M. Valpuesta2, José L. Carrascosa2, Oscar Llorca3, Jaime Martín-Benito2 and Juan Ortín1
1Department of Molecular and Cellular Biology, Centro Nacional de Biotecnología (CSIC), Darwin 3, 28049 Madrid, Spain and CIBER de Enfermedades Respiratorias (ISCIII).
2Department of Macromolecular Structures, Centro Nacional de Biotecnología (CSIC), Darwin 3, 28049 Madrid, Spain. 3Centro de Investigaciones Biológicas (CSIC), Ramiro de Maeztu 9, 28040 Madrid, Spain.

The genome of influenza viruses contain 8 virus ss-RNA segments of negative polarity that associate to the polymerase complex (PB1+PB2+PA) and nucleoprotein (NP) monomers to form ribonucleoprotein particles (vRNPs), which are responsible for virus transcription and replication. We have analysed the structure of biologically active polymerase-RNA template complexes, recombinant mini-RNPs and full-length virion RNPs by cryo-EM and 3D reconstruction. The structure of a recombinant RNP containing a 248 nt-long mini-replicon showed a nonameric NP ring with two monomers connected to the polymerase complex. In contrast, full-length virion RNPs showed a double helical structure with a minor and a major grove and revealed NP-NP interactions among the forward and backward NP chains that may be important for the biological activity of the RNPs. Docking the atomic structure of the NP and mutational analyses defined the interactions between the functional elements of the RNP. In addition, the structure of RNPs in intact virions was verified by cryo-electron-tomography and volume averaging. Altogether, these results provide the first model for a functional negative-stranded RNA virus RNP. To analyse the structure of the polymerase we generated polymerase-RNA template complexes by replication in vivo of a 46 nt-long micro-replicon. Their generation was strictly dependent on the polymerase activity, they contained mainly negative polarity viral RNA and transcribed and replicated efficiently in vitro. The structure of these polymerase-vRNA complexes was similar but distinct from that of the

077 **Biochemical and biophysical analysis of disctinctive human resiratory syncytial virus proteins: phospoprotein, M2-1 antiterminat**

Sebastián Esperante, Esteban Pretel and Gonzalo de Prat Gay
Fundación Instituto Leloir. IIBBA CONICET. Buenos Aires, Argentina.

Human respiratory syncytial virus (hRSV) is a worldwide distributed pathogen that infects most infants and elderly people. It shares several basic gene products with the paramyxoviridae family related to replication, attachment and fusion. With the aim of understanding fundamental, either comparative or distinctive, biochemical mechanisms, we tackled the characterization of: i) the phosphoprotein (P), the RNA polymerase cofactor; ii) the M2-1 transcription antiterminator, present only in pneumovirus, and iii) the non-structural protein NS1, a type I interferon antagonist, unique to RSVs. The P tetramer is highly stable with a modular unfolding coupled to dissociation. The M2-1 tetramer shares a strikingly similar stability (36.8 and 37.3 kcal/mol), but is highly dependent on pH. Both proteins interact with with a KD of 8 nM through a singular tetramer-tetramer interface. M2-1 bears an essential cys3-his1 zinc binding motif that can be found in Sendai and Ebola viruses and some eukaryotic transcription factors. We found that removal of the zinc atom leads to an Apo-M2-1 monomer, with secondary structure and stability identical to the tetramer. Dissociation is highly increased at pH 5.0 strongly suggesting that zinc removal, and therefore dissociation, is governed by the protonation of the histidine residue, indicative of an independent folding module with its non-specific RNA binding activity unaffected. Rather different solvent conditions cause irreversible self-oligomerization of NS1, leading to discrete stable and spherical species (NS1SOs). The convergence of these conditions, including a mild temperature change, suggest that N1SOs may accumulate in cells, where multiple conformational equilibria could be related to NS1 low binding specificity.

078 **A polymerase supercomplex within vesicular stomatitis virus**

Jeffery Hodges(1,2), Xiaolin Tang(1,2), Michael B. Landesman(1,2), John B. Ruedas(3), Anil Ghimire(1), Manasa V. Gudheti(4,7) , Jacques Perrault(3), Erik M. Jorgensen(5,7), Jordan M. Gerton(1,6) and Saveez Saffarian(1,2,7)
(1) Dept. of Physics and Astronomy, University of Utah
(2) Center for Cell and Genome Science, University of Utah
(3) Dept. of Biology, San Diego State University
(4) Vutara, Inc., Salt Lake City, UT
(5) Howard Hughes Medical Institute
(6) Dept. of Bioengineering, University of Utah
(7) Dept. of Biology, University of Utah

Negative sense non-segmented (NNS) RNA viruses package multiple copies of their polymerase complexes, which include a catalytic subunit L and a template-binding subunit P bound to a nucleoprotein-encapsidated RNA genome template (N-RNA). In the case of VSV, the bullet-shaped appearance of the virion results from tightly wound helical turns of the N-RNA template around a central cavity where 50 L and 400 P proteins are located, although their precise position within the cavity has not been defined. Using fluorescence photoactivatible localization microscopy (fPALM) and atomic force microscopy (AFM), we show that L and P proteins form a supercomplex starting from the blunt end of the virus and extending 57 ± 12 nm along the central cavity. Within this supercomplex, 4.5 L and 35 P proteins package per turn of the N-RNA helix. Together these observations suggest that there is significant (L-P)-(L-P) interactions within the virion and these interactions stabilize the supercomplex near the 5’ end of the genome during virus budding.

079 **Crystal structure of the N0-P complex of Nipah virus and of VSV provide new insights into the encapsidation mechanism**

Filip Yabukarski1, Cédric Leyrat1, Nicolas Tarbouriech1, Malene Ringkjøbing Jensen2, Martin Blackledge2, Rob Ruigrok1 and Marc Jamin1
1 - UMI 3265 UJF-EMBL-CNRS, Unit of Virus Host Cell Interactions, 6 rue Jules Horowitz, 38042 Grenoble, France
2 - UMR 5075 CEA-CNRS-UJF, Institut de Biologie Structurale, 41 rue Jules Horowitz, 38027 Grenoble, France

Encapsidation of newly synthesized viral RNA genomes and antigenomes in a helical homopolymer of nucleoprotein (N) is an essential step in the replication of non-segmented negative strand RNA viruses. For *Rhabdoviridae* and *Paramyxoviridae*, encapsidation relies on the continuous production of soluble RNA-free nucleoprotein (N0) in the form of a complex with the phosphoprotein (P), named the N0-P complex. In both families, this complex is formed by the interaction between the N-terminal region of P and the core of the N protein. We developed a strategy to reconstruct the N0-P complex from purified components using an N mutant deleted of its N-terminal arm (~ 20 aa) and a peptide of P containing the N0-binding site. For vesicular stomatitis virus (VSV), a rhabdovirus, and for Nipah virus (NiV), a paramyxovirus, we characterized the structure of the N-terminal region of P alone in solution and we solved the crystal structure of the reconstructed N0-P complex. This work demonstrates that the N-terminal region of P is globally disordered but contains transiently populated α-helices, and that it folds upon binding to the N0. The structure of the reconstituted N0-P complexes revealed how P prevents N0 from polymerizing and thereby from binding RNA. These structures also suggest mechanisms by which P stimulates initiation of RNA synthesis and controls encapsidation of the RNA genome. The structure of the NiV complex provides the first glimpse at the structure of the nucleoprotein of a *Paramyxovirinae*.

080 **Functional insights into measles virus NTAIL-PXD interaction using the biGene/biSilencing (biG/BiS) system**

Joanna Brunel1, Antoine Gruet2, Marion Dosnon2, Patricia Devaux3, Roberto Cattaneo3, Sonia Longhi2 et Denis Gerlier1
1CIRI, INSERM U1111, CNRS UMR5308, ENS Lyon, Université Lyon 1, CERVI, 21 Avenue Tony Garnier 69007 Lyon.
2CNRS et Aix-Marseille Université, Architecture et Fonction des Macromolécules Biologiques (AFMB), UMR 7257, Marseille, France
3 Mayo Clinic, Rochester MN, USA

The dynamic interaction of the C-terminal X domain (PXD) of P with the alphaMoRE located at the C-terminal of N (NTAIL) of each N subunit is proposed to allow the P tetramer to cartwheel along the helicoidal NC of paramyxovirinae. This ensures the regular displacement of the P-L polymerase complex during viral RNA synthesis. Mutations that possibly affect the dynamics of PXD/NTAIL interaction were introduced in PXD and tested in the virus infection context. The failure of P provided in trans to rescue measles virus whose endogenous P gene expression was silenced by RNAi led us to set up the bigene-bisilencing system "biG-biS". This consists in building recombinant viruses with a duplicated P gene, P1 and P2, with each gene copy being targeted by siRNA si1 and si2, respectively, so as to get selective expression of P1 or P2 in two independent cell lines constitutively expressing the corresponding shRNA. By allowing selective expression of natural P1, bi-P viruses with a wild-type phenotype were easily rescued and amplified in si2 cells. Infection of si1 cells allowed studying the functional impact of mutations introduced in P2. F497D substitution was deleterious for virus growth albeit tolerated when co-expressed with wild type P. F497A substitution resulted in a much slower virus growth. To circumvent the poor expression of PXD mutants in E. coli, the impact of these substitutions on the strength of interaction with NTAIL was explored using a mammalian luciferase-based protein complementation assay adapted for measuring monomeric and oligomeric interactions in cellula.

081 **A STRUCTURE FUNCTION ANALYSIS OF THE SENDAI VIRUS MATRIX-PROTEIN USING AN INTEGRATED SUPPRESSION COMPLEMENTATION SYTEM (ISCS)**

Geneviève Mottet-Osman and Laurent Roux
Department of Microbiology and Molecular Medicine, Faculty of Medicine, University of Geneva, Geneva Switzerland.

The Sendai virus (SeV) matrix protein M appears as one of the central organizers of viral assembly at the plasma membrane. It is proposed to bridgethe interactions of the nucleocapsid with the cytoplasmic tails of the glycoproteins.To get some more insights of the SeV M function, recombinant SeV viruses (rSeV) were constructed carrying the propertyi) to conditionally suppress the endogenous M protein and ii) to express a second copy of M, marked with an HA-tag, that was mutated in different motifs proposed to play a role in M function, namely late domains, actin binding domain or native structure stabilization domain. Serial N-ter and C-ter deletions were also produced. This integrated suppression complementation system (ISCS) allowed the evaluation of the importance the motifs/domains for the ability of M to support SeV particle production. This approach allowed us to characterize non-functional M proteins that were not incorporated into virus particles, even in the presence of wt-M. These were, of course, not able to complement for wt M suppression. Of the mutants that were still functional in that they were incorporated in virus particles, those that were still able to bind to nucleocapsids were more prone to complement for wt-M suppression, providing that they still bound to membrane fractions. In the end, the ISCS approach provided access to characterization of mutants in the context of the infection that could not have been analyzed otherwise

082 **Isolation and characterization of an N°-P complex of the Respiratory Syncytial Virus**

Marie Galloux, Gaëlle Gabiane, Jean-François Eléouët
INRA, Unité de Virologie et Immunologie Moléculaires UR892, F-78350 Jouy-en-Josas, France

Respiratory Syncytial Virus (RSV) is the leading cause of severe respiratory tract infections in young children and calves, however no human vaccine or antiviral drugs are still available. The viral polymerase complex (RdRp), which presents no equivalent in the cell, represents a target of choice for drug design.To this end, characterization of the structure and function of the proteins belonging to the RSV RdRp constitute a prerequisite. As for all non-segmented negative-strand RNA viruses the genome of RSV is always tightly bound to the viral nucleoprotein (N) and maintained as a helical N:RNA ribonucleoprotein (RNP) complex, which is used as a template for transcription and replication by the viral polymerase. The specific recognition of the RNP by the viral RNA polymerase (L) is mediated by the phosphoprotein (P). During replication of the viral genome, the newly synthesized (+) RNA intermediate antigenomes and (-) RNA genomes must be encapsidated by RNA-free nucleoprotein (N°). A second function of P is to interact with N and to act as an N-specific chaperone to maintain a pool of N°. However the N°-P complex of the RSV has never been isolated. We have recently generated a N recombinant protein mutated on two residues that are involved in the interaction with RNA. This N protein is i/ monomeric, ii/ RNA-free, and iii/ still interacts with P, suggesting that it could mimic the N°. We have also identified 2 sites of interaction of P with N°, at the N- and C-terminus of P.

abstracts

083 **Amino acid substitutions in human respiratory syncytial virus N protein affect differentially viral transcription and replication**

Ana Asenjo, Maria Luisa Navarro and Nieves Villanueva
Centro Nacional demicrobiologia (CNM).Instituto de Salud CArlos III(ISCIII)

Human respiratory syncytial virus, a pneumovirus from the Paramyxoviruses family, is the main cause of severe lower respiratory tract infections affecting babies and toddlers and inmunocompromised and old adults. After broad molecular and immunological studies of HRSV infections, two main conclusions have been reached: the best vaccine should be an attenuated virus and because the primoinfection is unable to induce long term protective inmunity, other reactives in addition to vaccines are needed for the virus health control. Among these reactives are the antiviral compounds. Our contribution to the developing of HRSV specific antivirals is to identify viral protein residues essential for developing its functions in the viral growth cycle. This information and that of the tridimensional structure viral proteins could be useful for designing specific antivirals against HRSV. In the paramyxoviruses , the N protein always enwrapped the viral RNA that acts as template for the synthesis of the different viral RNAs. This synthesis is carried out by the viral RNA dependent RNA polymerase (RdRp). N protein suffers different posttranscriptional modification as phosphorylation intyrosine (Y) at position 38. By locating N protein modified or in contact with the RNA residues, several of them have been found essential for viral transcription ,or replication or both, as it has been described for other mononegaviruses. The implication of these results on the existence of two RdRp one for transcription (RdRpT) and other for replication (RdRpR) should be discussed

084 **Labeling of influenza viruses with synthetic fluorescent and biotin-labeled lipids**

Natalia A. Ilyushina (1), Evgeny S. Chernyy (2), Elena Y. Korchagina (2), Aleksandra S. Gambaryan (3), Stephen M. Henry (4), Peter F. Wright (1), Nicolai V. Bovin (2)
(1) Geisel Medical School at Dartmouth, 1 Medical Center Drive, Lebanon, NH 03756, USA;
(2) Shemyakin Institute of Bioorganic Chemistry, Russian Academy of Sciences, Miklukho Maklaya 16/10, Moscow 117997, Russia;
(3) Chumakov Institute of Poliomyelitis and Viral Encephalitides, Russian Academy of Medical Sciences, Moscow Region 142782, Russia;
(4) Biotechnology Research Institute, AUT University, Private Bag 92006, Auckland 1142, New Zealand.

Direct labeling of virus particles is a powerful tool for visualization of virus-cell interactions. However, this technique involves chemical modification of viral proteins that affects viral biological properties. Here we describe an alternative approach of influenza virus labeling that utilizes Function-Spacer-Lipid (FSL) constructs that can be gently inserted into virus membrane and are optimized for integration into viral membranes. We assessed whether labeling with fluorescent, fluo-Ad-DOPE, or biotin-labeled, biot-CMG2-DOPE, probes has any deleterious effect on influenza virus hemagglutinin (HA) receptor specificity, neuraminidase (NA) activity, and replicative ability *in vitro*. Our data showed that both constructs did not significantly affect infectivity and affinity to sialyl receptors of the human and avian influenza A and B viruses. Neither constructs influenced NA activities of all the influenza viruses tested, except A/Puerto Rico/8/34 (H1N1) strain. N1 NA enzyme activity of this virus was reduced in ~82 % and ~52 % after labeling with fluo-Ad-DOPE and biot-CMG2-DOPE, respectively, which could be associated with the individual properties of the particular NA protein. FSL lipid-labeling provides a powerful new tool for studying early stages of influenza virus infection, such as virus attachment, endocytosis of the virus into the target cells and intracellular trafficking of the endocytosed virus. The features of the FSL constructs should make it possible to use labeled influenza virions to identify cells (such as dendritic cells, macrophages, B cells, and cells in the mucosa) that interact with the virus early in infection and assess which subsets of cells internalize/bind the virus *in vivo*.

085 **Disulphide-linked CM2 oligomers are required for efficient replication of influenza C virus**

Yasushi Muraki(1), Takako Okuwa(1), Toshiki Himeda(1), Seiji Hongo(2), Yoshiro Ohara(1)
1 Department of Microbiology, Kanazawa Medical University School of Medicine, Uchinada, Ishikawa 920-0293, Japan
2 Department of Infectious Diseases, Yamagata University Faculty of Medicine, Yamagata, 990-9585, Japan

CM2, the second membrane glycoprotein of influenza C virus, consists of 115 amino acids and is encoded by the RNA segment 6 (M gene) of the virus. CM2 possesses three conserved cysteines at residues 1, 6 and 20 in its extracellular domain, all of which are involved in the formation of disulphide-linked oligomers. To examine the effect(s) of CM2 oligomerization on influenza C virus replication, we generated rC1620A, a mutant recombinant virus in which all three cysteines on CM2 were substituted to alanines, and C1620A-VLPs, virus-like particles (VLPs) possessing the mutant CM2 protein. The rC1620A virus grew less efficiently than did the wild-type (rWT) virus in cultured cells. The amount of the genome incorporated into one-step grown rC1620A virions tended to be smaller than that into rWT, suggesting the involvement of CM2 oligomerization in the packaging process. Analyses of VLPs and VLP-infected cells showed that the amount of the genome (GFP-vRNA) present in C1620A-VLPs was approximately 31% of that in wild-type (WT-) VLPs, and the incoming genome (GFP-vRNA) from VLPs was less efficiently transported to the nucleus in the C1620A-VLP-infected cells than in the WT-VLP-infected cells, leading to reduced GFP expression in the C1620A-VLP-infected cells. The former and the latter findings suggest the involvement of CM2 oligomerization in the packaging and the uncoating processes, respectively. Taking these findings together, we conclude that disulphide-linked CM2 oligomers affect the packaging and the uncoating processes, facilitating efficient influenza C virus replication.

086 **Structure of the mumps virus phosphoprotein**

Robert Cox, Todd J. Green, Sangeetha , Champion Deivanayagam, Gregory J. Bedwell, Peter E. Prevelige and Ming Luo
University of Alabama at Birmingham

The phosphoprotein (P) is virally encoded by the Rhabdoviridae and Paramyxoviridae in the order Mononegavirales. P is a self-associated oligomer and forms complexes with the large viral polymerase protein (L), the nucleocapsid protein (N), and the assembled nucleocapsid. P from different viruses has shown structural diversities even though their essential functions are the same. We systematically mapped the domains in mumps virus (MuV) P and investigated their interactions with nucleocapsid-like particles (NLPs). Similar to other P proteins, MuV P contains N-terminal, central, and C-terminal domains with flexible linkers between neighboring domains. By pulldown assays, we discovered that in addition to the previously proposed nucleocapsid binding domain (residues 343-391), the N-terminal region of MuV P (residues 1-194) could also bind NLP. Further analysis of binding kinetics was conducted using surface plasmon resonance. This is the first observation that both the N- and C-terminal regions of a negative strand RNA virus P are involved in binding the nucleocapsid. Additionally, we defined the oligomerization domain (POD) of MuV P as residues 213-277 and determined its crystal structure. The tetrameric MuV POD is formed by one pair of long parallel α-helices with another pair in opposite orientation. Unlike the parallel orientation of each α-helix in the tetramer of Sendai virus POD, this represents a novel orientation of a POD where both the N- and C-terminal domains are at either end of the tetramer. This is consistent with the observation that both the N- and C-terminal domains are involved in binding the nucleocapsid.

087 **Location and structure of an antibody-binding site of the body domain of influenza A virus nucleoprotein**

Natalia Varich, Galina Sadykova, Prilipov Alexey, Kochergin-Nikitsky Konstantin, Kaverin Nikolai
D.I. Ivanovsky Institute of Virology, Moscow, Russia

Data on the amino acid positions recognized by monoclonal antibodies (MAbs) in influenza A virus nucleoprotein (NP) were reported in our previous publications and in reports of other authors. However, only the highly variable amino acid residues scattered in the 3D structure of NP were identified as important for antibody binding, so that no patterns of the architecture of antibody-binding sites could be identified. In the present studies a screening of amino acid residues surrounding an amino acid position identified earlier as a part of linear epitope have been performed. The presented data have identified the first minimal pattern of a site containing variable and conservative amino acid residues recognized by partially overlapping MAbs on a body domain in 3D structure of the influenza virus NP.

088 **Influenza virus non-structural protein NS1 cooperatively binds virus-specific (+)-strand RNA sequences**

Daniel MARC, Denis Soubieux
UMR1282 - Infectiologie et Santé Publique INRA - Centre de Tours, France

Non-structural protein NS1 of influenza viruses plays a major role in countering the interferon response of the host, and is involved in the metabolism of viral and cellular RNAs. Its multiple activities all require a functional RNA-binding domain. NS1 is generally thought to bind non-specifically to several viral and cellular RNAs, notably to double-stranded RNAs (dsRNAs). We asked whether NS1 could exhibit some sequence-specificity towards its RNA ligands, and performed an in vitro selection (SELEX) to isolate NS1-specific aptamers. We identified two virus-specific sequences that are characteristic of the viral RNAs of positive polarity. The first motif, AGCAAAAG, is strictly conserved at the 5'-end of all (+)-strand RNAs of influenzaviruses A. The second motif, UGAUUGAAG, is highly conserved in NS1-mRNA, 15 nucleotides downstream of NS1's stop codon. In addition, most of NS1-aptamers had one or two symmetrically positioned copies of the 5'-GUAAC / 3'-CUUAG double-stranded motif, which closely resembles the canonical 5'-splice site. We characterized the interaction of NS1 with its RNA-aptamers and showed that NS1's RNA-binding domain specifically recognizes both the sequence and structure of the virus-specific RNA-sequences. Cooperative binding of NS1's RNA-binding domain leads to its oligomerization on the bound RNA. This strong and intimate interaction suggests that NS1 activity towards viral RNAs is much more specific than previously thought.

089 **NMR reveals alpha-helical propensity in RSV P protein outside the oligomerization domain**

Safa Lassoued1, Marie Galloux2, Jenna Fix2, Carine Van Heijenoort1, François Bontems1, Jean-François Eléouët2, Christina Sizun1
1: Institut de Chimie des Substances Naturelles, CNRS UPR2301, 91190 Gif-sur-Yvette, France
2: Unité de Virologie et Immunologie Moléculaires (UR892), INRA, 78352 Jouy-en-Josas, France

The P protein of human Respiratory syncytial virus (hRSV) is an essential co-factor of the polymerase during replication and transcription. As compared to other Mononegavirales P proteins, hRSV P is rather short and does not seem to comprise domains with stable tertiary fold outside the central trypsin-resistant tetramerization domain, modeled in coiled-coil conformation (Llorente, 2006 & 2008). Although the regions at the N and C-termini of the oligomerization domain are predicted to be mainly intrinsically disordered, alpha-helical propensity was suggested in both (Simabucco, 2011). Here we took advantage of Nuclear Magnetic Resonance as a unique tool to investigate intrinsically disordered proteins and regions (IDRs) to analyze the secondary structure propensities of the N- and C-terminal regions of hRSV P, using backbone chemical shift analysis and 15N relaxation experiments. Data were measured with full-length P as well as several truncated forms, where the N- or C-terminal regions were deleted. Our results provide experimental evidence that the N- and C-terminal regions are indeed IDRs, but we also mapped two regions, located at the N-terminus of the protein and downstream of the tetramerization domain, that exhibit clear alpha-helical propensity. In contrast, the 10 residue long C-terminal stretch that was shown to bind to ribonucleoprotein complexes (Tran, 2007]) is completely unfolded. The identified regions are likely sites for molecular recognition of the other components of the hRSV polymerase complex and more particularly of the nucleoprotein, which is expected to display different binding modes, corresponding to the N°-chaperone or decapsidation functions of hRSV P.

090 **Characterization of the multiple Crimean-Congo Hemorrhagic Fever Virus Nucleoprotein interactions**

Jesica M. Levingston Mac leod1,3, Natalia Frías-Staheli4 and Adolfo García-Sastre1,2,3
1Department of Microbiology, 2Department of Medicine, Division of Infectious Diseases and 3Global Health and Emerging Pathogens Institute, Mount Sinai School of Medicine, New York, NY, USA.
4Center for the Study of Hepatitis C and Laboratory of Virology and Infectious Diseases, The Rockefeller University, New York, NY, USA.5

Crimean-Congo hemorrhagic fever virus (CCHFV) is a nairovirus, member of the family *bunyaviridae*, which infection often causes severe hemorrhagic fever in humans, with mortality rates above 30%. CCHFV is the most widespread among the tick-born human viral diseases and it is endemic in areas of Asia, Middle East and Africa. Its viral genome consists of three negative-sense RNA segments. The largest segment encodes a 448 kDa viral RNA-polymerase (L) and the Small segment encodes the Nucleoprotein (N). The Nucleoprotein of the bunyaviruses binds the viral RNA forming the Ribonucleoprotein (RNP) complex, subsequently; the RNA polymerase is attached to these structures allowing the initiation of the viral replication. The nucleoprotein also interacts with cellular proteins in critical process for the viral replication cycle, although the residues/regions implicated in these interactions within the nucleoprotein were hitherto unknown. By immunoprecipitation (IP) and immunofluorescence analysis, we mapped the regions within N protein implicated in RNA, actin and self binding. These results correspond with the previously shown importance of some of the residues within the carboxy terminal region for the formation of a RNA binding pocket or platform. In contrast, the central region (250-300) of N protein is required for the oligomerization and actin interactions. Furthermore, we described for the first time the interaction with the L protein in IP assays, and the importance of the amino and carboxy terminal regions within the RNA polymerase that might be necessary for the formation of the N-L complexes. The functional mapping of the N protein may improve the development of new CCHFV antiviral strategies.

091 **NiV N expression is repressed by hnRNP D wih its binding to NiV N 3'UTR**

Kimihiro Hino, Hiroki Sato, Akihiro Sugai, Masahiko Kato, Misako Yoneda, Chieko Kai *Laboratory Animal Research Center, Institute of Medical Science, The University of Tokyo.*

Nipah virus (NiV) is a non-segmented single-stranded negative-sense RNA virus belonging to the genus Henipavirus, family Paramyxoviridae. NiV causes acute encephalitis and respiratory disease in humans with high mortality, and poses a threat in southern Asia. The genomes of henipaviruses are about 18.2 kb (nt) in size, which is longer than those of other paramyxoviruses (around 15.4 kb nt). This difference is caused by the noncoding RNA region, particularly the 3' untranslated region (UTR), which occupies more than half of the noncoding RNA region. To determine the function(s) of the NiV noncoding RNA region, we investigated the effects of NiV 3'UTRs on reporter gene expression. The NiV N 3'UTR (nt 1-100) demonstrated strong repressor activity associated with hnRNP D protein binding to that region. Mutation of the hnRNP D binding site or knockdown of hnRNP D resulted in increased expression of the NiV N 3'UTR reporter. Our findings suggest that NiV N expression is repressed by hnRNP D through the NiV N 3'UTR binding, and demonstrated the involvement of post-transcriptional regulation in the NiV life cycle. To the best of our knowledge, this provides the first report of the functions of the NiV noncoding RNA region.

092 **Interactions between the Sendai virus HN glycoprotein and the matrix M protein**

Anastasia Shevtsova, Manel Essaidi-Laziosi, Laurent Roux
University of Geneva

Sendai virus encodes two glycoproteins HN and F. Incorporated into virus particles, both proteins are essential for virus fusion and entry into the cell. HN binds to the cellular receptor and F makes the fusion between the viral envelope and the cell plasma membrane. At late steps of the multiplication cycle, F plays an essential role in virus particle formation, in contrast to HN, which can be dispensable. In the cytoplasmic domain of HN glycoprotein, the motif SYWST is responsible for HN uptake in virus particles. We made the hypothesis that SYWST motif works by interacting with the matrix M protein. Pull down experiments using cytoplasmic domain of HN fused to a peptide which allows its biotinylation showed weak interactions with M protein in comparison with the F counterpart. We next took advantage of recombinant viruses harboring SYWST mutations that impaired HN uptake in virus particles to select revertants at secondary sites. Mutations in the M protein were observed and relevance of these mutations will be discussed.

094 **The Cys3-His1 Zinc binding motif of the hRSV M2-1 tetramer modulates its dissociation to folded apo-monomers.**

Sebastian Esperante, Maria Gabriela Noval, Tamara Antonella, and Gonzalo de Prat Gay *Fundación Instituto Leloir. - IIBBA CONICET*

The M2-1 protein of human respiratory syncytial virus (hRSV) functions as a transcription antiterminator by increasing polymerase processivity, thus enhancing readthrough of intergenic junctions. It is present only in pneumoviruses, namely hRSV and metapneumovirus. The hRSV M2-1 is a tight tetramer bearing a Cys3-His1 motif present in eukariotic transcription factors, which binds one zinc atom per monomer, and was shown to be essential for protein function by mutational analysis of the zinc coordinating residues. Using chemical perturbation, cross-linking, size exclusion chromatography, and dynamic light scattering, we showed that removal of the zinc atom leads to an Apo-M2-1 monomer. However, the secondary structure and stability of the apo-monomer is indistinguishable from the M2-1 holo-tetramer, indicating conservation of the native fold in the monomer. Thus, the complete exposure of its unique trp residue is not indicative of tertiary structure loss but is a sensitive reporter for metal removal and quaternary structure. Dissociation is much increased at pH 5.0 compared to pH 7.0 which srongly suggests that pH modulates zinc removal by histidine protonation and this, in turn, dissociation. Although no specific RNA sequence has been identified so far for M2-1, the monomeric apo form binds tRNA at least as well as the holo-tetramer. On the other hand, the monomer appears not to be competent for interacting with the phosphpoprotein P, the essential RNA polymerase cofactor. We discuss the results in connection with possible consequences in hRSV RNA transcription and genome replication.

095 **The Measles Virus Nucleoprotein Tail is Required for Packaging of Viral Polymerase Components into Nascent Particles**

Stefanie Krumm1, Richard Plemper1 2
1 Georgia State University
2 Emory University

The paramyxovirus polymerase complex consists of the P and L proteins that interact with N protein-encapsidated RNP for replication and transcription. N contains a core domain involved in RNA encapsidation and an ~100-residue C-terminal N-tail considered to mediate P-L binding to RNP for polymerization. N-tail of measles virus (MeV) is largely unstructured, but a terminal microdomain is implicated in P binding. To better understand the organization of MeV N and the role of N-tail sections upstream of this microdomain in polymerase activity and particle assembly, we subjected the protein to linker insertion mutagenesis and monitored N bioactivity in minireplicon assays. A central section of N-core and all sites tested in N-tail tolerated linker insertion. However, only N-tail accepted insertion of larger epitope tags upstream of the interaction microdomain in minireplicon assays and, after recovery of the corresponding recombinant MeV, the context of viral infection. This recombinant showed a 24-hour initial delay in replication, followed by wild type-like proliferation. Reinfection with progeny virus reproduced this growth profile, excluding viral adaptation during the lag phase. Monitoring the amplification kinetic of viral mRNA and genome after infection revealed a threshold-effect: delayed onset of primary transcription and replication, followed by wild type-like late-state polymerization. Characterization of purified mutant RNPs revealed reduced incorporation of P-L into virions. Our study identifies folding domains in the MeV N-core. Modifying the N-tail section has little effect on polymerase bioactivity, but a proper spatial organization of the tail is critical for efficient packaging of polymerase components into particles.

096 **Folding and conformational diversity of the non-structural NS1 protein from hRSV.**

Esteban Pretel, Mariana Gallo, Gabriela Camporeale and Gonzalo de Prat Gay
Fundación Instituto Leloir. - IIBBA CONICET

Human respiratory syncytial virus (hRSV) is the most important infectious agent responsible for pediatric respiratory disease worldwide. The virus encodes two non-structural proteins, NS1 and NS2 that are unique to RSV. These proteins are known to suppress type I interferon (IFN) response and signalling, and to interact with members of different pathways. Although NS1 is considered one of the main virulence factors of hRSV, the biochemical and biophysical knowledge is very scarce and there is no structure available. In this work, we describe a comprehensive analysis of the NS1 protein stability, folding, and conformational equilibria. Despite being a monomer, an important portion of the molecule is subjected to slow conformational motions as judged from NMR experiments. Under different conditions NS1 is able to self-oligomerize. Mild temperatures induce conformational changes displaying a highly cooperative transition. As a result, soluble spherical oligomers (SOs) with amyloid-like or repetitive ß-sheet type of structures were found. Conformational stability analysed by chemical perturbation showed a cooperative unfolding of the monomeric protein in a fully reversible process. In the case of NS1SOs, two transitions were observed indicating the presence of a native-like monomeric intermediate prior to unfolding.
Different solvent perturbations were remarkably coincident in the formation of ß-sheet enriched oligomer species with a secondary structure highly similar to those obtained alfer mild temperature treatment. Conformational complexity may well be related to NS1 function and binding promiscuity, expanding the possibilities of interactions in the different environments of the host cell.

097 **Probing conformational changes of the RSV F protein using conformation-specific antibodies and recombinant soluble F proteins**

Alan Rigter1,2, Ivy Widjaja1,2, Peter J.M. Rottier1, Bert Jan Haijema2, Cornelis A.M. de Haan1
1. Virology Division, Department of Infectious Diseases & Immunology, Faculty of Veterinary Medicine, Utrecht University, The Netherlands.
2. Mucosis B.V., Meditech Center, L.J. Zielstraweg 1, 9713 GX Groningen, The Netherlands.

Respiratory syncytial virus (RSV) is an important cause of respiratory tract disease. Currently, no licensed vaccine against RSV is available. The RSV F protein is a class I fusion protein that is cleaved by furin into F1 and F2. Homotrimers of F form the metastable pre-fusion structure. During infection, conformational changes in the F protein lead to the insertion of the hydrophobic fusion peptide into a host cell membrane. This fusion intermediate then refolds into the highly stable post-fusion structure. Although the post-fusion form of RSV F was shown to contain neutralizing epitopes, F protein preparations mimicking a pre-fusion form of RSV F probably expose more relevant epitopes. Therefore, we designed a set of recombinant soluble F proteins and evaluated their conformation and reactivity with (non)-neutralizing antibodies. Our data indicate that soluble F readily adopted the post-fusion conformation, the formation of which was not prevented by the addition of a GCN4 trimerization motif. Mutation of the furin cleavage sites or of heptad repeat B (HRB) prevented the formation of the post-fusion form and gave rise to different intermediate conformations. The pre-fusion conformation of F was only stabilized by the introduction of cysteine residues in HRB of GCN4-extended soluble F, which led to the formation of intermolecular disulfide bridges. The pre- and post-fusion forms of F clearly differed in their reactivity with neutralizing antibodies. Our results are in agreement with models in which the pre-fusion form of F undergoes consecutive conformational changes resulting in the formation of its post-fusion structure.

098 **Structural characterization of measles virus P oligomerization domain**

David Blocquel, Johnny Habchi, Eric Durand, François Ferron, Nicolas Papageorgiou and Sonia Longhi *CNRS et Aix-Marseille Université, Architecture et Fonction des Macromolécules Biologiques (AFMB), UMR 7257, Marseille, France*

The genome of Paramyxoviridae is encapsidated within a helical nucleocapsid by the N protein. The viral polymerase is recruited onto the nucleocapsid template by the P protein that serves as an essential polymerase cofactor. In Paramyxoviridae members, the P protein was shown to possess a modular organization, consisting in alternating intrinsically disordered and structured regions. The P proteins from members of the Paramyxovirinae subfamily are predicted to share a similar organization consisting in a large N-terminal disordered region (PNT) common to both P and V, and a C-terminal region (PCT) that is unique to the P protein. Previous computational studies showed that measles virus (MeV) PCT consists of a structured domain (PMD), predicted to be responsible for P multimerization, of a disordered linker and of a C-terminal alpha-helical X domain (XD). The exact oligomeric state of MeV P is not known, although it is thought to be tetrameric by analogy with the related Sendai virus (SeV). So far, structural data on Paramyxovirinae PCT are only available in the case of SeV. Here, using a combination of Small Angle X-ray Scattering (SAXS) and X-ray crystallography, we provide the first structural description of MeV PMD. Both biochemical and structural data consistently show that MeV PMD adopts a coiled-coil conformation in solution with an overall elongated shape consisting of an N-terminal structured moiety and of a disordered C-terminal appendage.

099 **The N-terminus of NEP of Influenza A viruses regulates the polymerase-enhancing function by backfolding to the C-terminu**

Peter Reuther, Linda Brunotte, Veronika Götz, Martin Schwemmle
Institute for Virology, Freiburg, Germany

Transmissions of highly pathogenic avian H5N1 viruses to humans pose a constant risk of a new pandemic. The lower temperature of the human upper respiratory tract represents a major hurdle for the establishment of infection by avian H5N1 viruses. Consistently, we observed an almost inactive H5N1 polymerase at 34°C after reconstitution in 293T cells, while a human adapted polymerase was not affected. Strikingly, the presence of NEP, harboring an adaptive mutation within its N-terminus, rescued the avian H5N1 polymerase activity at 34°C to levels observed at 37°C. The NEP-dependent rescue of polymerase activity at lower temperature was also observed after infection of A549 cells with recombinant H5N1 virus encoding the human-adapted NEP as compared to the same virus expressing avian NEP. Analysis of deletion mutants revealed that the polymerase-enhancing function of NEP resides in the C-terminus and that removal of the last three amino acids abrogates this activity. We therefore speculated that the adaptive mutation in the N-terminus regulates accessibility of the C-terminus by reducing the affinity between N- and C-terminus. Subjecting the protein to an intra-molecular conformation assay using split-luciferase technology, we show that human-adapted NEP retains an open conformation compared to avian NEP at lower temperatures. We therefore propose that the N-terminus of NEP exerts an autoregulatory function by backfolding to the C-terminus. In this model, adaptive mutations in NEP allow the protein to "open up" preferentially at low temperature.

100 **Lyssavirus targeting of STAT proteins: a critical determinant of pathogenicity and potential therapeutic target**

Linda Wiltzer 1, Aaron Brice 1, Satoko Yamaoka 2, Florence Larrous 3 , Herve Bourhy 3, Danielle Blondel 4, David Jans 5, Naoto Ito 2, Gregory Moseley 1
1 Viral Pathogenesis Laboratory, Dept. of Biochemistry and Molecular Biology, Monash University, Clayton, VIC, Australia
2 Laboratory of Zoonotic Diseases, Faculty of Applied Biological Sciences, Gifu University, Gifu, Japan
3 Institut Pasteur, Unit Lyssavirus Dynamics and Host Adaptation, Paris, France
4 Laboratoire de Virologie Moléculaire et Structurale, CNRS, Gif sur Yvette, France
5 Nuclear Signaling Laboratory, Dept. of Biochemistry and Molecular Biology, Monash University, Clayton, VIC, Australia

The lyssaviruses are a genus of zoonotic viruses including rabies virus (RABV) and Australian bat lyssavirus that cause neurological disease with ~100% case-fatality rates, resulting in >55,000 human deaths/year. Lyssaviruses inhibit interferon (IFN)-mediated immune signaling through the activity of viral IFN-antagonist P-proteins. We have shown that IFN-antagonism by P-proteins involves diverse mechanisms dependent on interactions with host-cell STAT1/STAT2 proteins, nuclear transport factors, and the microtubule cytoskeleton (1-4). These mechanisms are thought to be essential to pathogenicity, thus representing potential targets for new vaccine strains/antivirals. However, their importance to virulence has not been demonstrated in vivo. Using an approach combining single-cell imaging, immune signaling assays, reverse genetics and a unique in vivo pathogenicity model, we identified specific sequences of P-protein underlying interactions with STATs, nuclear transporters and microtubules. Through detailed mapping studies, we generated P-proteins with specific defects in these interactions, but which retain functions in viral replication, enabling the production of viable recombinant viruses impaired for particular IFN-antagonism mechanisms. Using these strains, we have been able to delineate the importance of these mechanisms in infection *in vivo* for the first time, demonstrating critical contributions to the development of lethal neurological disease in animals. Importantly, our recent work identified point mutations able to increase the IFN-sensitivity of an invariably lethal RABV strain, rendering it apathogenic in vivo. These mutations are being used to generate new potential animal vaccine strains.

101 **Marburg virus structural protein VP24 acts as an activator of Nrf2 pathway by targeting cellular Keap1.**

A.Page1, SP.Reid2, M.Mateo1, K.Nemirov1, V.A.Volchkova1, A.C.Shurtleff2, P.Lawrence1, A.Baule1, O.Reynard1, M.Ottmann1, V.Lotteau3, R.K.Thimmulappa4, S.Biswal4, S.Bavari2, V.E.Volchkov1
1.Molecular basis of viral pathogenicity, CIRI, INSERM U1111, Université de Lyon,Lyon, France
2.United States Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Frederick, MD 21702-5011, USA
3.IMAP team, CIRI,INSERM U1111, Université de Lyon, Ecole Normale Supérieure de Lyon, Lyon, France
4.Department of Environmental Health Sciences, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD 21205, USA

Marburg virus (MARV) causes hemorrhagic fever in man characterized by massive virus replication, dysregulated inflammation and an extremely high fatality rate. In this study we have discovered a previously unknown function of MARV VP24 protein and reveal the mechanism by which this virus activates an antioxidant signaling pathway. We demonstrate that VP24 binds Kelch-like-ECH-associated protein-1 (Keap1), a negative regulator of an important cellular transcription factor; Nuclear factor erythroid-derived-2 (Nrf2). Binding of VP24 to Keap1 releases Nrf2 from Keap1 control, allowing its translocation into the nuclei and activation of a panel of cytoprotective genes implicated in cellular responses to environmental insults and regulation of inflammation. Furthermore we show that knockout of Nrf2 in mice inhibits MARV replication resulting in resistance to infection compared to wild-type animals. Persistent activation of the Nrf2-pathway evidently favor prolonged survival of MARV-infected cells despite massive virus replication and may explain dysregulation of inflammatory responses during infection.

102 **Identification of an Endocytic Calcium Channel TPC2 as a Novel Host Factor of Ebolavirus Infection**

Yasuteru Sakarai, Andrey A. Kolokoltsov, Robert A. Davey
Texas Biomedical Research Institute, San Antonio, Texas, USA

Ebolavirus (EBOV) is a negative stranded RNA virus that causes hemorrhagic fever with high mortality in humans and the other primates. While promising candidates have been proposed, there are currently no vaccines or antiviral drugs licensed for clinical use. The virus has a complex, yet poorly understood infection mechanism and this knowledge is important for developing new therapies. Previously, we reported the involvement of host calcium signaling pathways in EBOV infection and have been working to understand the roles of the components and also how calcium, itself, is involved in the infection. While most other viruses utilize calcium related pathways during or after genome replication, we found that EBOV requires calcium to be present before genome replication begins, indicating a role in virus entry. Here we report that several known L-type calcium channel inhibitors, such as the FDA approved drug verapamil, strongly blocked EBOV infection. However, we found that the classical L-type channel proteins were not necessary for infection. Instead, a novel endocytic calcium channel, TPC2, which has been shown sensitive to L-channel inhibitors, is the key host factor. Among other data, TPC2-specific siRNAs suppressed EBOV infection and Ned19, a chemical antagonist of TPC2, inhibited virus entry. We will discuss the mechanistic role of TPC2 in controlling EBOV entry into host cells and the potential for using the inhibitors as therapy for EBOV disease. This work was supported by funding from the Ewing Halsell Foundation.

103 **Epigenetic Factors in Arenavirus Infection: Roles for Glycosylation and Phosphorylation**

Michael Buchmeier, Cyrille Bonhomme, Kristeene Knoop, Lydia Bederka *Department of Molecular Biology and Biochemistry, University of California, Irvine*

The arenaviruses glycoprotein (GPC) is glycosylated at 11 conserved N-glycosylation sites. We have used recombinant Lymphocytic choriomeningitis virus (rLCMV) featuring additions or deletion of these N-glycans to investigate their role in protein expression, tropism and immune recognition. N-glycosylation at sites T87 and S97 was necessary to rescue rLCMV. Three of the 9 successfully rescued mutant rLCMV (G104N, S398A, and T234A) fell under selective pressures in either epithelial, neuron, or macrophage cells to conserve their N-glycan sites. Of the 7 N-glycan deletion mutants, 5 of these led to variable modifications in viral fitness and cell tropism towards either neurons or macrophages. Early studies of LCM Virus showed that nucleoprotein (NP) was phosphorylated during the process of replication. We have re-examined this old observation in light of recent structural models of the arenavirus NP. Three sites of phosphorylation in the LCMV NP have been confirmed. These confirmed phosphorylation sites mapped to the extended portion of Helix α5, which is disordered when RNA is bound. To determine how these sites might regulate replication and transcription of the viral genome we made recombinant virus (rLCMV) containing point mutations at each of these confirmed phosphorylation sites. One site, LCMV NP T206 is conserved in all mammalian Arenaviruses, and this residue is required for formation of replication transcription complexes in the cytoplasm. NP T206A is functional in transcription, however, in the presence of the matrix protein, Z, this mutant demonstrates almost complete loss of transcription and replication, and a loss of infectivity in rLCMV.

104 **The Intracellular Cargo Receptor ERGIC-53 is Required for the Production of Infectious Arenavirus Particles**

Joseph P. Klaus1 , Philip Eisenhauer1, Joanne Russo1, Anne Mason1, Danh Do1, Benjamin King1, Douglas Taatjes1, Cromwell Cornillez-Ty2, Jonathan Boyson1, Chunlei Zheng3, Lujian Liao2, John R. Yates III2, Bin Zhang3, Bryan A. Ballif1, and Jason Botten1
1University of Vermont, Burlington, VT 05405, USA
2The Scripps Research Institute, La Jolla, CA 92037, USA
3Genomic Medicine Institute, Lerner Research Institute of Cleveland Clinic, Cleveland, OH 44195, USA

Arenaviruses and hantaviruses cause severe and often fatal diseases in humans. Effective antivirals or FDA-approved vaccines do not exist for these pathogens and little is known regarding host proteins required for their propagation. To address this deficiency we have comprehensively identified human proteins that interact with the glycoproteins (GPs) of a prototypic arenavirus and hantavirus. We show that ERGIC-53 - an intracellular cargo receptor that facilitates the anterograde transport of a limited number of glycoprotein ligands in the early exocytic pathway, including the blood coagulation factors (F)V and FVIII - has a highly conserved association with arenavirus and hantavirus GPs. The molecular basis for ERGIC-53's interaction with arenavirus GPs is unique from its previously characterized cellular ligands; only the C-terminal region of ERGIC-53's carbohydrate recognition domain (CRD) is required for the interaction whereas ERGIC-53's ability to oligomerize, traffic, or bind mannose, Ca2+, or multiple coagulation factor deficiency 2 protein are not. ERGIC-53 trafficking is strikingly altered during arenavirus infection; it traffics to sites of virus assembly and budding at the plasma membrane and is incorporated into virions. Finally, ERGIC-53 is required for the propagation of both Old and New World arenaviruses; in its absence, GP-containing virions form, but are noninfectious due to a defect in their ability to undergo early replication events, namely attachment to, and/or entry into, host cells. In summary, we have identified a new class of ERGIC-53 ligands, a novel, lectin-independent basis for ERGIC-53's association with these ligands, and a critical role for ERGIC-53 in arenavirus replication.

105 **Deciphering the glycosylome of dystroglycanopathies with a haploid genetic screen for Lassa virus cell entry**

Matthijs Raaben1, Lucas T. Jae2, Moniek Riemersma3, Ellen van Beusekom4, Vincent A. Blomen2, Arno Velds2, Ron. M. Kerkhoven2, Jan E. Carette5, Haluk Topaloglu6, Peter Meinecke7, Marja W. Wessels8, Dirk J. Lefeber3, , Hans van Bokhoven4, Thijn R. Brummelkamp2 and Sean P.

1Department of Microbiology and Immunobiology, 77 Avenue Louis Pasteur, Harvard Medical School, Boston, MA 02115, USA.

2Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.

3Department of Neurology, Institute for Genetic and Metabolic Disease, Radboud University Medical Centre, Nijmegen, The Netherlands.

4Department of Human Genetics, Nijmegen Centre for Molecular Life Sciences, Radboud University Medical Centre, Nijmegen, The Netherlands.

5Department of Microbiology and Immunology, Stanford University School of Medicine, 299 Campus Drive, Stanford, CA 94305, USA.

6Hacettepe University Children's Hospital, 06100, Ankara, Turkey.

7Institut für Humangenetik, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany.

8Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, The Netherlands.

Lassa virus (LASV), a member of the family Arenaviridae, is a highly pathogenic agent causing hemorrhagic fever in humans with several hundred thousand infections per year in Africa and thousands of deaths annually. Alpha-dystroglycan (α-DG), an ubiquitous receptor for extracellular matrix proteins, serves as the cell entry receptor for LASV. α-DG is extensively modified by sugar chains, including an unusual O-linked glycan, which is essential for its function as a LASV receptor. Interestingly, defects in α-DG glycosylation cause Walker-Warburg syndrome (WWS), a severe hereditary multi-organ dystrophy. At least eight proteins are critical to glycosylate α-DG, but many of the genes mutated in WWS patients remain unknown. Here, we performed a haploid genetic screen for LASV cell entry using a recombinant vesicular stomatitis virus expressing the LASV glycoprotein. This screen identified several *Registration, Abstract Submission & Payment form- page 2 of* host factors essential for LASV cell entry, including all known WWS genes and five additional genes involved in glycosylation (e.g. *TMEM5, B3GALNT2, B3GNT1, SLC35A1, and SGK196*). Validation experiments showed that these genes are critical for the post-translational modification of α-DG. We sequenced the coding exons of *TMEM5* and *SGK196* in a panel of 28 patients with severe dystroglycanopathy, not carrying mutations in any known WWS gene. Two families with patients that carried homozygous mutations in *TMEM5* were identified and one patient with a heterozygous mutation in *SGK196* was discovered in another family. Our findings accentuate the complexity of α-DG glycosylation and point out new genes defective in inherited dystroglycanopathies as well as potential targets for antiviral therapy.

106 **Nairobi Sheep Disease Virus (NSDV) blocks the JAK-STAT-signalling pathway at the level of the Janus kinases**

Barbara Holzer, Michael D Baron
The Pirbright Institute

Nairobi sheep disease virus (NSDV) is highly pathogenic in sheep and goats, causing acute haemorrhagic gastroenteritis and abortion. NSDV (known as Ganjam virus (GV) in India) is a member of the genus Nairovirus within the family Bunyaviridae and is closely related to Crimean-Congo hemorrhagic fever virus, which causes a similar severe disease in humans. Many viruses have evolved systems to overcome innate immune responses, and we have previously shown that NSDV/GV is able to block the STAT1 and 2 phosphorylation induced by type I and II interferons (IFN). In this study we have shed light on how the virus is interfering with the Jak-STAT signalling pathway. Expression of the individual viral proteins revealed that the N-terminal half (L1-1757) of the viral RNA-dependent polymerase is able to block IFN-induced phosphorylation of STAT1. However, no physical interaction of STAT1 or STAT2 with L1-1757 could be detected. We could show that NSDV/GV is able to block Jak1 and Tyk2 phosphorylation in infected cells upon induction with IFNβ or IFNγ. Immunoprecipitation studies carried out with FLAG-tagged Jak1 and Tyk2 showed an interaction between those proteins and L1-1757. In addition, we could show that Jak1 autophosphorylation was inhibited by L1-1757. Interestingly, the enzymatic activity of the Ovarian Tumour-like protease (OTU) domain in L1-1757 was not required for the block in autophosphorylation of Jak1 or the block of IFN-induced STAT1 phosphorylation. Our data suggest that NSDV/GV blocks the IFN action pathway by targeting the tyrosine kinases of the Janus (JAK) family, Jak1 and Tyk2.

107 **Analysis of the NSs protein of the newly emerged Schmallenberg virus**

Gerald Barry (1), Mariana Varela (1), Marco Caporale (1,2), Frauke Seehusen (4), Maxime Ratinier (1), Esther Schnettler (1), Ilaria M. Piras (1, 3), Melanie McFarlane (1), Wolfgang Baumgärtner (4), Alain Kohl (1), Massimo Palmarini (1)
(1) MRC-University of Glasgow Centre for Virus Research, Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom.
(2) Istituto G. Caporale, Teramo, Italy
(3) Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari, Sassari, Italy
(4) Department of Pathology and Center of Systems Neuroscience, University of Veterinary Medicine, Hannover, Germany

Schmallenberg virus (SBV) is an orthobunyavirus that emerged in Europe in late 2011 and is now widespread in Europe. SBV causes a mild disease in adult cattle and sheep but it has been associated with outbreaks of congenital defects in offspring, often leading to abortion of the foetus. SBV is an arbovirus, believed to be transmitted by biting midges Using a synthetic approach, we developed a reverse genetics system for the rapid rescue and genetic manipulation of SBV and also an experimental animal model of infection that has allowed us to study SBV pathogenesis. We found that a SBV deletion mutant lacking the non-structural NSs protein (SBVΔNSs) is less virulent in mice than wild type SBV. Attenuation of SBV virulence depends on the inability of SBVΔNSs to block cellular transcription and protein production by inhibiting RNA polymerase II function and not being able to block IFN production from virus infected cells. The SBV NSs protein also appears to manipulate apoptotic pathways that may be central to disease manifestation. Collectively these data suggest that the SBV NSs protein has similar functions to the NSs protein of other bunyaviruses although important differences do exist.

108 **Fragile X Mental Retardation Protein Stimulates Ribonucleoprotein Assembly of Influenza A Virus**

Zhuo Zhou, Yang Guo, Lili Zhao1, Jingfeng Wang1, Xue Jia Qinghua Xue, Sheng Cui, Qi Jin, Jianwei Wang, Tao Deng *Institute of Pathogen Biology, Chinese Academy of Medical Sciences*

The viral ribonucleoprotein (RNP) of influenza A virus consists of the viral RNA polymerase complex (PB1, PB2 and PA) bound to a genomic RNA segment that is coated with multiple copies of nucleoprotein (NP). The RNPs perform viral RNA transcription and replication in the nucleus, which require various interplays between host factors and the RNP components. By a cellular transcriptional profiling-based siRNA screen, we find that Fragile X Mental Retardation Protein (FMRP) is required for influenza virus replication. We show that FMRP supports influenza virus replication and directly targets viral RNA synthesis machinery. We further demonstrate that FMRP transiently associates with viral RNP and stimulates viral RNP assembly through interacting with viral nucleoprotein (NP). Furthermore, we find that the KH2 domain of FMRP mediates its association with NP. A point mutation (I304N) in the KH2 domain, identified from a Fragile X syndrome patient, disrupts the FMRP-NP association and destroys the ability of FMRP in viral RNP assembly. We conclude that FMRP is a critical host factor used by influenza viruses to facilitate viral RNP assembly. Our observation reveals a mechanism of influenza virus RNA synthesis and provides insights into FMRP functions.

109 **The Splicing Factor Proline-Glutamine Rich (SFPQ/PSF) Is essential in Influenza Virus Polyadenilation**

Sara Landeras-Bueno, Núria Jorba, Maite Pérez-Cidoncha and Juan Ortín
Centro Nacional de Biotecnología (CSIC), Darwin 3, Madrid and CIBER de Enfermedades Respiratorias (ISCIII)

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. 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. These results indicate that SFPQ/PSF is a host factor essential for influenza virus transcription that ncreases 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.

110 **VIRAL PROTEINS AND RIG-I LIKE RECEPTOR INTERACTION**

Maria T Sanchez-Aparicio (1), Juan Ayllon (1), Adolfo Garcia-Sastre (1,2,3)
(1)Department of Microbiology, (2)Department of Medicine, Division of Infectious Diseases, and (3)Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, New York, NY, 10029, USA.

The host innate immune system acts as the first line of defense to prevent viral invasion. Viral products are detected through several classes of pathogen recognition receptors (PRR), including the cytosolic RIG-I like receptors (RLRs). Once activated, RIG-I interacts with polyubiquitin chains generated by TRIM25 and binds MAVS, leading to the production of type I IFN. Using Bimolecular Fluorescence Complementation we have analyzed the subcellular localizations and interactions among RIG-I, MAVS and TRIM25. Many viruses have developed multiple mechanisms to interfere with the RLR pathway and evade the innate immune response in infected cells. Here we show in living cells the impact of different viral proteins in the protein-protein interactions among cellular components of the RLR pathway. NS1, the main IFN antagonist encoded by influenza A viruses, prevents the formation of the complexes RIGI-MAVS and TRIM25/TRIM25, but does not have any effect on the complex RIG-I/TRIM25. These complexes are localized in different compartments in the host cell. The NS3/4A protease of hepatitis C virus changes the localization of the complexes RIGI-MAVS and MAVS-MAVS. V protein of Nipah virus interacts directly with RIG-I and TRIM25, participating in RIG-I/RIG-I and RIG-I/TRIM25 complexes. Experiments are in progress to further analyze the localization and the effects of the viral IFN antagonist proteins on these complexes in the host cell.

111 **B-1 is a porter to lead influenza viral ribonucleoprotein complexes to microtubules**

Atsushi KAWAGUCHI, Kyosuke NAGATA
Department of Infection Biology, Faculty of Medicine, University of Tsukuba

De novo synthesized RNAs are under the regulation of multiple post-transcriptional processes by a variety of RNA-binding proteins. The influenza virus genome consists of single-stranded RNAs and exists as viral ribonucleoprotein complexes (vRNP). After the replication of vRNP in the nucleus, vRNP is exported to the cytoplasm and then reaches the budding site beneath the cell surface mediated by Rab11a-positive recycling endosomes along microtubules. However, the regulatory mechanisms of post-replicational processes of vRNP immediately after the nuclear export are largely unknown. Here, we identified, as a novel vRNP-interacting protein, Y-box binding protein-1 (YB-1), a cellular protein that is involved in regulation of cellular transcription and translation of cellular mRNAs. YB-1 translocated to the nucleus from the cytoplasm and accumulated in PML nuclear bodies in response to influenza virus infection. vRNP assembled into the exporting complexes with YB-1 at PML nuclear bodies. Using YB-1 knockdown cells and in vitro reconstituted systems, after nuclear export YB-1 was shown to be required for the interaction of progeny vRNP with microtubules around the centrosome, which functions as a microtubule organization center (MTOC), where Rab11a-positive recycling endosomes were located. Further, we also found that over-expression of YB-1 increases the production of progeny virions in an Rab11a-dependent manner. Taken altogether, we propose that YB-1 is a porter that leads vRNP to microtubules from the nucleus and put it on the vesicular trafficking system. Currently, analyses to clarify the effect of YB-1 on the centrosome function are ongoing.

112 **Interaction between human parainfluenza virus type 2 V protein and an antiviral host factor, tetherin**

Machiko Nishio1, Keisuke Ohta1, Masato Tsurudome2, Daniel Kolakofsky3
1: Department of Microbiology, Wakayama Medical University,
2: Department of Microbiology and Molecular Genetics, Mie University Graduate School of Medicine,
3: Department of Microbiology and Molecular Medicine, University of Geneva School of Medicine

Tetherin, also known as BST-2/CD317/HM1.24, is a cellular protein that restricts budding of a number of enveloped viruses including HIV-1, HIV-2, SIV and Ebola virus. These viruses have evolved specific countermeasures to overcome restriction by tetherin, e.g., the membrane protein of HIV-1, the glycoproteins of HIV-2, SIV and Ebola virus. Human parainfluenza virus type 2 (hPIV-2) belongs to the family *Paramixoviridae* and also one of enveloped viruses. However, little is known about the relationship between hPIV-2 and tetherin. The accessory V protein of hPIV-2 is multifunctional and is reported to induce STAT protein degradation and the blockage of TLR7/9-dependent signaling. Recently, we have found that V interacts with tetherin using immuno-precipitation, which leads to hypothesis that V is a candidate for tetherin antagonist although it is not a membrane protein unlike other viral antagonists. P protein of hPIV-2, whose N-terminal domain is common to the V, did not bind to tetherin, indicating that the C-terminal V-specific region interacts to tetherin. Tetherin is a type II trans-membrane protein containing of an N-terminal cytoplasmic tail, an extracellular domain, and a C-terminal glycosyl phosphatidylinositol membrane anchor (GPI). The deletion of GPI disrupted the interaction with V, suggesting that GPI is critical for the binding to V. Since V is the first reported protein that binds to GPI of tetherin, hPIV-2 might antagonize it with a different mechanism from those by other enveloped viruses.

113 **Extrinsic apoptosis subversion by RSV P in macrophages**

Yuko Nakamura-López (1), Nicolás Villegas-Sepulveda (2), Beatriz Goméz (3)
1. Consejo Estatal para la Prevención y Control del Sida-Centro Ambulatorio para la Prevención y Atención del Sida (COESIDA-CAPASITS), San Bartolo Coyotepec, Oaxaca, México, C.P. 71256. yuko@unam.mx
2. Departamento de Biomedicina Molecular, CINVESTAV, México, D.F. 07360
3. Virology Lab, Facultad de Medicna, UNAM, México, D.F. 04510

Respiratory Syncytial Virus (RSV) is associated with high morbidity and mortality due to bronchiolitis and pneumonia in children with sequels of chronic hyperreactivity, suggesting persistence of RSV. P and determined cell viability and caspase-8 activity. N with the viral protein from M N). RSV proteins involved in the apoptotic process were searched by comparing sequence between viral and cell proteins involved in apoptotic process. The effect in the apoptotic process by viral proteins was evaluated through transfecting M P and mock-infected macrophages (M P). We extended these observations to determine whether the extrinsic apoptotic process was also altered and if viral proteins participate in changes of the apoptotic process. To this end, we analyze comparatively the expression of several components of the DISC complex in M Previously we reported that intrinsic apoptosis pathway is subverted in a murinemacrophage cell line persistently infected with RSV (M RSVP. Data suggest that suppression of extrinsic pathway is related to enhance cIAP2 and the expression of RSV P protein acting as a vFLIP. These findings are important to understand the intracellular and viral genes involved in subversion of apoptosis by RSV persistence in macrophages. RSVP) survives 20% more than mock-transfected macrophages, and the caspase-8 activity were not observed in M transfected with RSV P protein (M N, though caspase-8 was not activated. P sequence showed 38% similarity to caspase-8 death domain. After staurosporine treatment, M P expression of TNF-alpha, its receptor and anti-apoptotic protein cIAP2 were enhanced with respect to M In M *Acknowledgment.* This work is supported by CONACYT- grant 78862 and by GlaxoSmithKline Mexico

abstracts

113.a **Paradoxical effect of nitric oxide on respiratory syncytial virus replication in a persistently infected murine macrophage cell**

Kenya M. Maldonado-Pérez, Carlos Santiago-Olivares, Beatriz Gómez
Laboratorio de Virología, Depto. de Microbiología y Parasitología, Facultad de Medicina, Universidad Nacional Autónoma de México, México, D.F.

The production of nitric oxide (NO), a highly reactive free radical, acts as an important component of the host immune response in several viral infections and is considered as an antiviral effector of some DNA and RNA virus. The aim of this study was to examine the effects of endogenous and exogenous nitric oxide on the level of respiratory syncytial virus replication in a persistently infected macrophage cell line (MφP). Confirmation of RSV persistence in MφP was monitored by determining cell viral antigen expression and the percentage of RSV-positive cells through flow cytometry assays, extracellular virus infectivity determined by TCID50/ml. NO synthesis was indirectly determined by NO2- production through Griess assay, the number of RSV genome copies (RSVc) was measured through amplifying RSV N protein by qRTPCR with the Path –RSV-ÁD Prime Design Kit, Southampton, United kingdom. . Macrophages endogenous NO production was stimulated by treatment with inactivated non-typeable Haemopihlus influenza (NTHi), and NO production inhibited with LNAME, competitor substrate of inducible synthasa II. Exogenous NO was produced with SNAP a nitric oxide donor. Endogenous NO in MφP reduced RSVc in a factor of 1.94 and increased nitrate concentration in 1.83 factor. Inhibition NO synthesis by LNAME, increased RSVc and reduced nitrite concentration in 2.47 and 0.62 factors respectively. The data suggests that NO has in MφP antiviral effect. Surprisingly in the same cell line exogenous NO production had an opposite effect, RSVc increased in a 185 times, implying that NO viral RSV polymerase activity and/or its synthesis were stimulated.

114 **Tracking of fluorescently labeled Newcastle Disease Viruses in living cells**

Angela Römer-Oberdörfer, Anja Röder, Stefan Finke, and Thomas C. Mettenleiter
Institute of Molecular Biology, Friedrich-Loeffler-Institut, Federal Research Centre for Animal Health

Expression of green fluorescent protein (GFP) by recombinant Newcastle Disease Virus from an additional gene has already been described. However, for virus tracking in living cells autofluorescent label of specific viral proteins is required. Therefore, the hemagglutinin-neuraminidase protein (HN) was fused at the carboxy terminus with GFP. Two different recombinant NDV on the basis of lentogenic NDV Clone 30 genome were rescued, one of them contains a polybasic amino acid sequence at the cleavage site of the fusion protein, resulting in virus which replicates well in a number of tissues. Characterization of both viruses demonstrates that labeled virus recombinants are similar in their replication characteristics to their unlabeled precursor, despite an increase of the HN protein by 230 amino acids of GFP. The incorporation of GFP labeled HN into virus particles could be confirmed by western blot analyses of purified virions. Infection of QM9 cells with both autofluorescent NDV demonstrated that the intracellular transport rate of HN protein from perinuclear regions to the plasma membrane is unaffected, whereas the number of new infected cells within 17 hours of observation was increased after infecting with NDV which carried the polybasic amino acid sequence at the F cleavage site. These data confirm the results of replication kinetics and the known causal connection between amino acid sequence at the cleavage site and infectivity.

115 **Hsp70 protein positively regulates Rabies Virus infection**

Xavier Lahaye, Aurore Vidy, Baptiste Fouquet, Danielle Blondel
CNRS, UPR 3296 Virologie Moléculaire et Structurale, 91198, Gif sur Yvette, France

Hsp70 chaperone plays a central role in multiple processes within the cells, including protein translation, folding, intracellular trafficking and degradation. This protein is implicated in the replication of numerous viruses. We have shown that rabies virus infection induced the cellular expression of Hsp70 that accumulated in Negri bodies-like structures where viral transcription and replication take place. In addition, Hsp70 is present in both nucleocapsids purified from infected cells and purified virions. Hsp70 has been shown to interact with the nucleoprotein N. Down regulation of Hsp70, using specific chaperon inhibitors such as quercetin or RNA interference resulted in a significant decrease of the amount of viral mRNAs, viral proteins and virus particles. These results indicate that Hsp70 has a proviral function during rabies virus infection and suggest that Hsp70 is involved in at least one stage(s) of the viral life cycle such as viral transcription and/or translation and/or viral production . The mechanism by which Hsp70 controls viral infection will be discussed.

116 **CELL-SURFACE RECEPTOR USAGE OF OCOZOCOAUTLA DE ESPINOSA VIRUS**

Yíngyún Caì[1], Steven Mazur[1], Shuǐqìng Yú[1], Lián D ng[2], Krisztina Janosko[1], Téngfēi Zhāng[1], Marcel A. Müller[3], Sina Bavari[2], Peter B. Jahrling[1], Sheli R. Radoshitzky[2], and Jens H. Kuhn[1]
1 Integrated Research Facility at Fort Detrick, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Fort Detrick, Frederick, Maryland, USA;
2 United States Army Medical Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland, USA;
3 Institute of Virology, University of Bonn Medical Centre, Bonn, Germany

Ocozocoautla de Espinosa virus (OCEV) is a novel uncharacterized and uncultured arenavirus from Mexico's Chiapas State. Chiapas State experienced multiple viral hemorrhagic fever epidemics in the past, which led to the hypothesis that OCEV may have been their cause. Using gammaretrovirion-like particles pseudotyped with OCEV's glycoprotein or control arenavirus glycoproteins, we demonstrated that the New World clade B arenavirus receptor TfR1 functions also as the cell-surface receptor for OCEV. However, whereas OCEV pseudotypes could use TfR1 from mammals of multiple species, human TfR1 could not function as an interacting partner. In accordance, OCEV glycoprotein-pseudotyped particles were able to transduce several mammalian cell lines efficiently, but could not transduce more than 60 tested human cell lines. Furthermore, the introduction of TfR1 residues from a nonhuman mammal TfR1 ortholog into human TfR1 turned the latter into an efficient OCEV receptor. Expression of the nonhuman mammal TfR1 ortholog or of the chimeric TfR1 in human cells made them permissive for particle transduction. Together, these results suggest that the currently circulating OCEV variant is not a human virus, and therefore not a cause of viral hemorrhagic fever.

117 **Deciphering the antiviral activity of the human Mx system in a transgenic mouse model**

Christoph Deeg, Pascal Mutz, Carsten Kallfass, Cindy Nürnberger, Sébastien Soubies and Peter Staeheli *Department of Virology, University of Freiburg, Freiburg, Germany*

Mx proteins are interferon (IFN)-induced GTPases that confer antiviral activity in many vertebrates. Mouse Mx1 accumulates in the nucleus of IFN-treated cells and mediates antiviral activity in vitro and in vivo against orthomyxoviruses, such as influenza A and Thogoto viruses. Human MxA is a cytoplasmic protein that exhibits an even broader antiviral activity in cultured cells. However, to date, its in vivo potential remained poorly investigated. To fill this knowledge gap, we created a new mouse line that carries the entire human Mx locus as a transgene. We found that high levels of MxA were induced in cultured embryonic fibroblasts from transgenic mice upon treatment with IFN-α. Further, high levels of MxA were found in most organs of transgenic mice at 24 hours post treatment with recombinant IFN-α but not in organs of untreated animals, indicating faithful regulation of the human Mx locus in our transgenic mouse. Challenge experiments demonstrated that our transgenic mice are well protected from lethal infections with Thogoto virus and highly pathogenic avian influenza A virus strains. Interestingly, protection against influenza A virus strains of human origin, such as the H1N1 strains A/PR/8/34 and A/WSN/33 or the H3N2 strain A/HK/68, was only moderate. These results provide strong indirect evidence that the Mx locus plays an important role in influenza A virus protection of humans. They further indicate that human influenza viruses can largely overcome this barrier.

118 **The host-cell transcription machinery is a target for influenza virus polymerase-induced degradation that contributes to viral**

A. Rodriguez, R. Alfonso, C. Llompart & A. Nieto
Centro Nacional de Biotecnologia, Centro de Investigaciones Biomédicas en Red de Enfermedades Respiratorias (Ciberes).

Infection with non-attenuated influenza viruses induces profound alterations in the host-cell transcription machinery including degradation of the RNA polymerase II (RNAP II). Besides that, we have observed a concomitant degradation of CHD6, a chromatin remodeler that modulates host-cell transcription. The viral polymerase triggers both, RNAP II and CHD6 degradation in a proteasome-independent manner and interestingly, CHD6 degradation is also observed *in vivo* in the lungs of influenza virus infected mice that correlates with the increase of viral titers in the lungs. Reverse-genetics experiments have indicated the role of PA and PB2 subunits on RNAP II degradation and here we have characterized that changes at positions 504 of PB2 together with 550 of PA in attenuated viruses confer the ability for RNAP II degradation and conversely, these changes in non-attenuated viruses abolish its degradation capacity. The introduction of these mutations in a 2009 pandemic strain, A/California/07/09 (CAL) virus, abolishes its 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 indicate that influenza virus induces a degradative process upon transcription-related host-factors that might contribute to its virulence and confirm the role of PA and PB2 subunits in the degradation process.

119 **Phospholipase (PLC) gamma 1 signaling plays a subtype-specific role in cell entry of influenza A virus**

Liqian Zhu, Vikram Verma, Naveen Kumar, Nishi R. Sharma, and Yuying Liang
Department of Veterinary and Biomedical Sciences, University of Minnesota, Twin Cities, MN 55108. USA.

Influenza virus infection causes acute lung disease that can lead to severe and deadly complications and is responsible for annual epidemics and occasional pandemics with high morbidity and mortality. Novel antiviral drugs are in urgent need due to the rapid emergence of drug-resistant viral variants against current antiviral medications. Host-based therapeutics are receiving increasing attention as an alternative approach due to their broad-spectrum therapeutic potential and expected low frequency of emergence of drug resistant viruses. Our laboratory studies the biological roles and functional mechanisms of host signaling pathways in the influenza viral life cycle and evaluates the therapeutic potentials of the respective inhibitors of cell signaling pathways in treating influenza virus infection (Kumar et al., J Virol 2008; Kumar et al., J Virol 2011; Kumar et al., AAC 2011). We have found that a specific receptor tyrosine kinase TrkA plays important roles in multiple replication steps of various strains of influenza virus and that TrkA inhibitor strongly blocks viral replication in vitro and in vivo. We have also identified for the first time the subtype-specific interplay of host PLC-γ1 signaling and H1N1 virus in the early virus entry step. The functional mechanisms of TrkA and PLC-γ1 signaling pathways in the influenza viral replication and their potential applications as new antiviral therapeutics will be discussed. In summary, our studies provide important insight into the virus-host interactions during influenza viral infections, and demonstrate that host signaling pathways may have a general or subtype-specific mechanism in the replication of different influenza viral strains.

120 **Isolation and Characterization of Hokkaido Virus, Genus Hantavirus**

Takahiro Sanada1, Yuka Ozaki1, Takahiro Seto1, Momoko Nakao1, Saasa Ngonda1, Kumiko Yoshimatsu2, Jiro Arikawa2, Kentaro Yoshii1, Ikuo Takashima1 and Hiroaki Kariwa1
1Lab. of Public Health, Dept. of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine, Hokkaido University
2Dept. of Microbiology, Graduate School of Medicine, Hokkaido University

Hantaviruses belong to the Bunyaviridae family and cause two severe human illnesses, hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). We isolated Hokkaido virus (HOKV), one of the hantaviruses using a new cell line, MRK101, derived from a kidney of gray red-backed vole (Myodes rufocanus bedfordiae), the natural host of HOKV. To characterize the isolated HOKV strain, Kitahiyama128/2008 in more detail, we analyzed the viral growth of HOKV in VeroE6 and MRK101cells as well as the sequences of S, M, and L segments of the virus. In MRK101 cells, the expression of HOKV N protein was confirmed at 14 days post inoculation (dpi) and the level of progeny virus gradually increased until 14 dpi. In contrast, no HOKV propagation was observed in VeroE6 cells. On the other hand, Puumala virus (PUUV) Samara 94 strain propagated both in MRK101 and VeroE6 cells. The nucleotide and amino acid sequences of HOKV were most closely related to PUUV (Identities in S segnemt, 81.9–83.8% nt and 94.7–95.8% aa) which causes HFRS. Phylogenetic analysis based on the nucleotide sequence of the coding region of the S, M, and L segments supported the close relationship between HOKV and PUUV. This is the first report of the hantavirus which propagates in a cell line that originated from the natural host but not in VeroE6 cells. Analyzing the reason for the inability of HOKV propagation in VeroE6 cells would provide us important information such as the host specificity of hantavirus.

121 **Relative cytopathogenesis of RSV in nasal epithelial cell cultures from infant cohorts with histories of severe and mild RSV.**

Hong Guo-Parke1, Olivier Touzelet1, Isobel Douglas2, Rémi Villenave1, Liam G. Heaney1, Peter V. Coyle3, Michael D. Shields1,2 and Ultan F. Power1
1Centre for Infection & Immunity, School of Medicine, Dentistry & Biomedical Sciences, Queens University Belfast, Belfast, Northern Ireland, UK, BT9 7BL; 2The Royal Belfast Hospital for Sick Children, Northern Ireland;
3The Regional Virus Laboratory, Belfast Trust, Belfast, Northern Ireland.

One to 3% of infants are hospitalised with RSV-induced bronchiolitis or pneumonia annually. The vast majority of infected infants, therefore, do not suffer severe disease. However, little is known about the relative behaviour of RSV in airway epithelial cells, the principle targets for RSV infection, from those that suffered severe or mild disease. To help address this, we developed a model of RSV infection based on well-differentiated paediatric primary nasal epithelial cells (WD-PNECs). The cells were derived from bilateral brushings of the medial aspect of the inferior turbinate from cohorts of infants with clinical histories of severe or mild RSV infections, respectively, in an ethically approved and consented study. WD-PNECS from each cohort were indistinguishable in terms of pseudostratification, ciliated and goblet cell content and tight junction integrity. RSV BT2a (clinical isolate) infection of WD-PNECs from both cohorts was restricted to apical ciliated cells but did not cause noticeable damage to the cultures over 6 days. Virus growth curves were similar in WD-PNECS from both cohorts. Syncytia were also evident on the apical surface of both. CXCL10, TRAIL and IL-29 secretions were significantly increased and to similar levels in RSV-infected cultures from both cohorts. RSV infection did not stimulate matrix mettaloproteinase (MMP) 1, 2, 3, 7, 9, 10, 12 and 13 secretions. Interestingly, however, endogenous MMP1 secretion levels were significantly higher in cultures derived from the mild cohort. Our data suggest that RSV interaction with airway epithelium may not be the determining factor in disease outcomes following RSV infection.

122 **ANP32B is a Nuclear Target of Hendra Virus Matrix Protein**

Sebastian Neumann1, Axel Karger1, Ann-Kristin Henning1, Linda Kwasnitschka, Anne Balkema-Buschmann, Günther Keil1, Stefan Finke1
1 Institute of Molecular Biology, Friedrich-Loeffler-Institut
2 Institute of Institute of Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut

Membrane envelopment and budding of NSVs is mainly driven by viral matrix (M) proteins. In addition,several M proteins are also known to be involved in host cell manipulation. Knowledge about the cellular targets and detailed molecular mechanisms, however, is poor for many M proteins. For instance, Nipah Virus (NiV) M protein trafficking through the nucleus is essential for virus release, potential nuclear targets of NiV M, however, have not been identified. To identify cellular interactors of henipavirus M proteins, tagged Hendra Virus (HeV) M proteins were expressed in transfected cell cultures and M-containing protein complexes were isolated by affinity purification. The composition of isolated protein complexes was analyzed by nLC-MALDI-TOF/TOF mass spectrometry and cellular ANP32B (acidic leucine-rich nuclear phosphoprotein 32 family, member B) was identified, suggesting that ANP32B directly or indirectly interacts with HeV M protein. ANP32B is involved in various cellular processes and could represent a target to affect cellular gene expression, apoptosis regulation and Exportin-1 (Crm1) dependent mRNA export. As ANP32B is involved in nuclear export, we tested whether nuclear shuttling of M was affected by ANP32B. Indeed, over-expression of ANP32B led to nuclear accumulation of M, whereas Exportin-1 dependent transport of Rabies virus P protein was not affected. From these data we conclude that ANP32B indeed is a specific nuclear target of HeV M and that ANP32B may participate in HeV M protein nuclear shuttling and/or in host cell manipulation by affecting cell survival or specific mRNA transport processes.

123 **High-content siRNA screen to identify cellular factors required for infection of Crimean-Congo hemorrhagic fever virus**

Olena Shtanko (1),Raisa Nikitina (2),Alexander Chepurnov (2),Robert Davey (1)
(1) Texas Biomedical Research Institute, San Antonio, TX 78227, USA.
(2) Institute for Clinical Immunology, Novosibirsk, 630047, Russia.

Crimean-Congo hemorrhagic fever virus (CCHFV) is a tick-borne bunyavirus causing outbreaks of severe hemorrhagic disease in humans, with a fatality rate approaching 30%. The virus is endemic to much of Eastern Europe, the Middle East, Asia, and Africa, although recent studies have detected CCHFV in ticks collected in Spain, indicating an expanding geographic distribution. The high lethality of CCHFV and the lack of approved countermeasures require work to be performed in a biosafety level 4 (BSL-4, P4) laboratory, hampering research of the pathogen. To date, little is known about host factors facilitating CCHFV infection. Here, we describe the first high-throughput small interfering RNA (siRNA) screen to identify cellular proteins required for entry and replication of fully infectious CCHFV. To perform this work, we generated a recombinant virus expressing the red fluorescent protein mKate2 (CCHFV-mKate2). Mammalian cells grown in 384-well plates were transfected with the druggable genome library of siRNA (26,600 siRNA targeting 6,650 genes) and infected with CCHFV-mKate2 in our BSL-4 laboratory at the Texas Biomedical Research Institute. Infected cells were imaged and counted by high-content analysis. Host genes important for infection were identified using the Redundant siRNA Activity Analysis Tool (Novartis), and gene networks were modeled by Ingenuity Pathway Analysis software. Endocytic regulators, kinases, phosphatases, and potential receptor proteins for CCHFV infection were identified. We will discuss novel aspects of this work and implications of our findings for drug development. This work was supported by HDTRA1-12-1-0002 FRBAA09-6H-2-0043 and Douglass and Ewing Halsell Foundations.

124 **Host mTORC1 signaling regulates Andes virus replication**

Shannon McNulty, Michael Flint, Stuart Nichol, Christina Spiropoulou
Viral Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA

Hantavirus pulmonary syndrome (HPS) is a severe respiratory disease characterized by pulmonary edema, with fatality rates of 35 to 45%. Disease occurs following infection with pathogenic New World hantaviruses, such as Andes virus (ANDV), which targets lung microvascular endothelial cells. During replication, the virus scavenges 5'-m(7)G caps from cellular mRNA to ensure efficient translation of viral proteins by the host cell cap-dependent translation machinery. In cells, the mammalian target of rapamycin (mTOR) regulates the activity of host cap-dependent translation by integrating amino acid, energy, and oxygen availability signals. We initially investigated whether inhibitors of the mTOR pathway could reduce hantavirus infection. We found that treatment with the FDA-approved rapamycin analogue temsirolimus (CCI-779) blocks ANDV protein expression and virion release but not entry into primary human microvascular endothelial cells. This effect was specific to viral proteins, as temsirolimus treatment did not block host protein synthesis. We confirmed that temsirolimus targeted host mTOR complex 1 (mTORC1) and not a viral protein, as knockdown of mTORC1 and mTORC1 activators but not mTOR complex 2 components reduced ANDV replication. Additionally, primary fibroblasts from a patient with tuberous sclerosis exhibited increased mTORC1 activity and increased ANDV protein expression, which were blocked following temsirolimus treatment. We also shown that ANDV glycoprotein Gn colocalized with mTOR and lysosomes in infected cells. Together, these data demonstrate that mTORC1 signaling regulates ANDV replication and suggest that the hantavirus Gn protein may modulate mTOR signaling during infection.

125 **Differential recognition of arenavirus glycoproteins and cellular substrates by subtilisin kexin isozyme 1/site 1 protease**

Dominique J. Burri1, Joel Ramos da Palma1, Nabil G. Seidah2, Giuseppe Zanotti3, Laura Cendron4, Antonella Pasquato1, and Stefan Kunz1

1 Institute of Microbiology, University Hospital Center and University of Lausanne, Lausanne, Switzerland

2 Laboratory of Biochemical Neuroendocrinology, Clinical Research Institute of Montreal, Montreal, Canada (Affiliated to the University of Montreal)

3 Department of Biomedical Sciences, University of Padua, Padua, Italy

The arenaviruses are an important family of emerging viruses including several causative agents of severe hemorrhagic fever in humans that represent serious public health problems. A crucial step of the arenavirus life cycle is maturation of the envelope glycoprotein precursor (GPC) by the cellular subtilisin kexin isozyme-1 (SKI-1)/site-1 protease (S1P). Proof-of-concept studies with peptide-derived and small molecule inhibitors pinpointed SKI-1/S1P as a promising target for anti-viral therapeutic intervention. However, considering the importance of SKI-1/S1P for normal physiology, the development of inhibitors that specifically block processing of viral GPCs but not cellular substrates is a primary goal. Using a combination of molecular modeling and structure-function analysis, we provide evidence for striking differences in the molecular recognition of viral GPCs and cellular substrates by SKI-1/S1P. Immature forms of SKI-1/S1P show normal activity towards cellular substrates but are impaired in processing of viral GPCs. Moreover, Old World and Clade C New World arenaviruses contain a characteristic aromatic "signature residue" at P7 in the GPC, which is recognized by residue Y285 of SKI-1/S1P, distal of the catalytic triad. The data suggest that during co-evolution with its mammalian host, GPCs of Old Worldand Clade C New World viruses expanded the molecular contacts with SKI-1/S1P beyond the classical four amino acid recognition sequences and currently occupy an extended binding pocket.

126 **Viral dsRNA detection by the RIG-I Like Receptors family**

Jessica Guerra, Stéphanie Anchisi, Dominique Garcin

Dpt. Microbiology and Molecular Medicine, School of Medicine. Geneva Switzerland.

The innate immune system is the first line of defense that all organisms have to sense and fight incoming pathogens, thanks to the presence of specific receptors: Pattern Recognition Receptors (PRRs) that discriminate between self and non-self in the host. RIG-I like receptors (RLRs) are a family of proteins that specifically detect RNA viruses in the cytoplasm of infected cells. Their activation ends up in the production of IFNβ and inflammatory cytokines, leading to the establishment of an anti-viral state. Over-production of IFNβ could be deleterious for the organism; therefore RLRs are tightly regulated by different mechanisms and at different levels. It is reported that the spliced variant form of RIG-I (RIG-I SV) functions as negative regulator of the signaling cascade, whereas to date the function of the third member of the family (LGP2) is still unclear. Interestingly the kind of RNA that both RIG-I SV and LGP2 could bind is unknown as well as possible cellular partners. Thus the aim of this work was to elucidate the contribution of the RNA binding capacity and proteinprotein interaction to their function. LGP2 and the ΔCARDs form of RIG-I were tested for their ability to: 1) interfere with RIG-I in the IFNβ production; 2) bind RNA. We could demonstrate that both of them negatively affect RIG-I signaling, upon different stimuli, leading to a reduction in IFNβ induction. Additionally we showed their binding to 5'ppp dsRNA pointing to a competition mechanism, however further investigations are needed to shed light into the regulation of RLRs family.

127 **Dissecting the molecular interaction of influenza A virus RNA polymerase and host RNA polymerase II.**

Mónica Martínez-Alonso, Narin Hengrung, Lauren Turrell, Frank Vreede, Ervin Fodor *Sir William Dunn School of Pathology, University of Oxford.*

The viral RNA polymerase is at the core of the transcription and replication complex of influenza virus, and viral transcription is functionally linked to transcription by cellular RNA polymerase II (Pol II). The viral polymerase interacts with the C-terminal domain (CTD) of Pol II (which consists of 52 heptad repeats of the conserved consensus sequence YSPTSPS) only when the CTD is phosphorylated in Ser5 (initiating form of Pol II). This interaction may be required for the synthesis of viral mRNA *in vivo*. However, it is unclear if this interaction is direct or indirect and whether viral RNA is involved. To address these questions we developed an *in vitro* binding assay to characterise the interaction between the viral polymerase and Pol II. We incubated synthetic Pol II CTD mimic peptides consisting of four heptad repeats (unphosphorylated or phosphorylated at Ser2 or Ser5) with a viral polymerase purified from mammalian cells and observed specific binding when Ser5 in the CTD is phosphorylated and a short vRNA is co-expressed with the viral polymerase. We observed similar results with a viral polymerase expressed in insect cells and bound to synthetic 5' and 3' ends of the vRNA promoter. Moreover, all three types of viral RNA could be co-immunoprecipitated with Pol II from 293T cells infected with influenza A/WSN/33 virus. Together, these results suggest that the interaction between the viral polymerase and initiating Pol II is direct and hint at a role of viral RNAs in this interaction.

128 **African Henipavirus surface proteins promote viral entry, cell fusion & cytopathic effects in Human, Simian & Bat cell lines**

Philip Lawrence1, Jan-Felix Drexler2, Victor Max Corman2, Beatriz Escudero-Perez1, Valentina Volchkova1, Marcel Müller2, Christian Drosten2 & Viktor Volchkov1
1 - CIRI, INSERM U1111, Lyon, France
2 - Institute of Virology, University of Bonn Medical Centre, Bonn, Germany

Throughout history, the emergence of new infectious diseases of animal origin has caused considerable problems for human health. Bats have been shown to serve as reservoirs for an array of infectious agents, including Henipa-, Filo-, Corona- and Lyssaviruses. Although Henipaviruses are traditionally limited to Pteropus bats of South Asia, the recent discovery of distinct viral clades in phylogenetic relation to Henipaviruses in diverse bat species in five African countries raises the possibility that these highly-pathogenic viruses may not be as geographically restricted as previously thought. In the absence of isolated infectious African Henipavirus, we have undertaken experiments with the viral entry proteins F and G from a representative African strain (M74a) with the aim of determining if this virus presents the same broad cross-species tropism as its Henipa relatives. When the M74a entry proteins are cross-expressed with those from Nipah virus, the M74a G protein is able to substitute for the NiV G protein but with delayed fusion kinetics for a variety of cell lines from different species. Co-expression of M74a G and F proteins in simian and host bat cell lines results in rapid cytopathic effects. Pseudotyped-MLV GFP reporter infection assays were used to evaluate the capacity of these viral surface proteins to permit entry into target cells. Entry mechanisms of the African Henipavirus will be discussed further. Results suggest that this virus could be expected to show a similar cell tropism to that of the other Henipa viruses and therefore present a risk for zoonosis.

129 **Mutual Antagonism between Ebola virus VP35 and PACT**

Priya Luthra1, Parameshwaran Ramanan2,3, Chad Mire4, Carla Weisend5, Yoshimi Tsuda5, Benjamín Yen1, Daisy W. Leung2, Thomas W. Geisbert4, Hideki Ebihara5, Gaya. K. Amarasinghe2, Christopher F. Basler1
1Dept. Microbiology, Mount Sinai School of Medicine, New York, NY 10029
2Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO 63110
3Biochemistry Graduate Program, Roy J. Carver Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University, Ames, IA 5001
4Galveston National Laboratory, Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX
5Laboratory of Virology, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, Montana, USA.

RIG-I-like receptors (RLR) are activated by viral RNA displaying a 5'-ppp motif and induce type I IFN responses to control viral replication. Recent studies show that cellular dsRNA binding protein PACT can also activate RIG-I. To counteract such antiviral pathways, some viruses, including Ebola virus (EBOV), encode proteins that antagonize RLR signaling. Here, we show that EBOV VP35 inhibits PACT-induced RIG-I ATPase activity in a dose-dependent manner. Co-immunoprecipitation of RIG-I with PACT is disrupted by WT VP35, but not by VP35 mutants unable to bind PACT. In addition, PACT-VP35 interactions impair VP35-polymerase interaction, diminishing viral RNA synthesis and modulating EBOV replication. However, PACT-deficient cells are defective in IFN induction and are insensitive to VP35 function. These data support a model where VP35-PACT interaction plays a critical role in determining the outcome of EBOV infection and host innate immune responses.

130 **Residue 57 of the Marburg Virus VP40 Matrix Protein Determines Resistance to Human Tetherin**

Alicia Feagins, Christopher Basler *Icahn School of Medicine at Mount Sinai*

Marburg viruses, members of the Filoviridae family, cause severe hemorrhagic fever in humans and non-human primates. The Ravn (RAVV) and Ci67 (MARV) strains of Marburg virus were recently adapted to mice. During adaptation, mutations accumulated throughout the viral genome with several occurring in the gene encoding VP40. VP40, the viral matrix protein, drives Marburg virus budding. RAVV and maRAVV VP40s bud effectively from Hepa1.6 cells (a mouse liver cell line) but only RAVV VP40 buds from Huh7 cells (a human liver cell line). Transfecting Hepa1.6 cells with either RAVV or maRAVV VP40 and increasing amounts of human tetherin revealed RAVV VP40 to be more resistant to the effects of tetherin than maRAVV VP40. Subsequent analysis revealed residue 57 to be important for VP40 resistance to human tetherin as there was a significant decrease in budding from Hepa 1.6 cells
transfected with a RAVV VP40 V57A mutant and increasing amounts of human tetherin when compared to RAVV VP40. Hepa1.6 cells transfected with a maRAVV VP50 A57V mutant and increasing amounts of tetherin had a modest increase in budding at lower concentrations of tetherin when compared to maRAVV VP40. The RAVV VP0 V57A and maRAVV VP40 A57V mutants however, maintained their ability to bud in Hepa1.6 cells transfected with increasing amounts of mouse tetherin. Taken together, the data suggests that amino acid residue 57 determines RAVV and maRAVV VP40 resistance to human tetherin but not mouse tetherin and that VP40 budding can be restricted by tetherin in a host cell-dependent manner.

abstracts

131 **Borna disease virus X protein: a new tool against neurodegenerative disorders?**

Marion Szelechowski 1, Alexandre Bétourné 1, Yann Monnet 2, Anne Thouard 1, Jean-Michel Peyrin 3, Stéphane Hunot 2, Daniel Gonzalez-Dunia 1
1 - Inserm UMR 1043, Centre de Physiopathologie de Toulouse-Purpan (CPTP), CHU Purpan, Toulouse
2 - Institut du Cerveau et de la Moelle épinière (ICM), La Pitié-salpétrière, Paris
3 - Université Pierre et Marie Curie (UPMC), Jussieu, Paris

Control of cellular apoptosis by virus-encoded products is a strategy often used by viruses to limit cellular damage and allow persistence in their host. Given the poor renewal capacity of neurons, it is even a key element for persistent neurotropic viruses. Among them, Borna disease virus persistence in the central nervous system of infected animal involves expression of a small 10 kDa mitochondrial protein called X. This observation led to the tantalizing hypothesis that the X protein may be endowed with neuroprotective properties. Here, using an innovative oriented primary neuronal culture based on microfluidics, we demonstrate that X protein is also neuroprotective in vitro against toxins that target the mitochondrial respiratory chain, even when expressed alone independently of the viral context. We show that the presence of X protein in the mitochondria network of neurons is sufficient to prevent the mitochondrial hallmarks of oxidative stress, i.e., ROS overproduction, membrane potential shutdown and cytochrome C release. We will also present clues of the underlying molecular mechanisms. Finally, we demonstrate that X protein expression in vivo in the nigrostriatal pathway is able to significantly reduce neuronal loss in murine models of Parkinson's disease. Based on these finding, it is tempting to speculate that the X protein could represent a new lead for the development of novel therapies against neurodegeneration.

132 **Length of the NS1 linker region determines pathogenicity of a highly pathogenic H5N1 influenza A virus**

Mirco Schmolke 1, Benjamin G Hale 2, Rong Hai 1, Jianqiang Ye 3, Balaji Manicassamy 1, Daniel R Perez 3, Adolfo García Sastre 1
1 Mount Sinai School of Medicine, Department of Microbiology, New York, NY, USA
2 University of Glasgow, Institute of Infection, Immunity and Inflammation, Glasgow, UK
3 University of Maryland, Department of Veterinary Medicine, College Park, MD, USA

The non structural protein 1 (NS1) of influenza A viruses (IAV) is a major viral pathogenicity factor. It is a multifunctional protein that blocks host cell mRNA maturation, splicing, activates PI3K signaling and interferes with RIG-I dependent recognition of viral RNA by the cytosolic RNA sensor RIG-I. NS1 is composed of a N-terminal RNA-binding domain (RBD), a central effector domain (ED) and a C-terminal unstructured tail domain. RBD and ED are connected by a flexible linker region, which presumably allows the two domains to arrange in different 3D conformations, depending on the cellular context and cellular or viral encoded interacting proteins /RNA. Interestingly, in some H5N1 influenza A NS1s from human cases this linker region is lacking 5aa. We were curious to see if this deletion is a species specific adaptation and tested the viral replication and pathogenicity in mammalian and avian host model systems.We show that reintroduction of these deleted amino acids can indeed impact pathogenicity of influenza A/Viet Nam/1203.

133 **The tetraspanin proteins, CD81 and CD9, promote efficient influenza A virus budding through interactions with HA and NA**

Yuhong Liang(1), Megan L. Shaw (1,2)
1 Department of Microbiology,Icahn School of Medicine at Mount Sinai
2 Graduate School of Biomedical Sciences, Icahn School of Medicine at Mount Sinai

Tetraspanins are members of a transmembrane glycoprotein superfamily and most are broadly expressed in human cells. They play a general role in organizing membrane protein complexes and CD81 and CD9 in particular are well known regulators of membrane fusion events. Tetraspanin proteins have been described to be involved at different stages of the life cycle for several viruses e.g. HIV-1, HCV. For influenza virus we have shown that CD9 and CD81 are incorporated into influenza virions and that CD81 is required during the viral entry process. To further characterize the role of these host proteins in the influenza viral replication cycle, we examined influenza A and B virus replication in cells depleted of CD9, CD63 and CD81 using RNAi knockdown or Nin CD81 over-expressing HepG2 cells. We find that CD9 and CD81, but not CD63, are required for optimal influenza A virus growth in tissue culture. In contrast, optimal influenza B virus growth does not require these host factors. We also examined influenza virus-like particles (VLPs) released from cells either over-expressing CD9, CD63 and CD81 or depleted of these tetraspanins. We find that overexpression of tetraspanins CD9 and CD81 (but not CD63) in producer cells enhances VLP generation, whereas RNAi knockdown of these two proteins reduces VLP budding. The positive regulatory effect of CD81 is observed with several influenza A virus subtypes. With co-immunoprecipitation, we find that CD81 interacts with influenza A virus HA (H1, H2, H3 and H5) and NA (N1, N2) but not influenza B virus HA, NA. Overall our data suggest that certain tetraspanins play an important role during the influenza A virus life cycle, and particularly at the budding step where interactions with the viral glycoproteins promotes production of virus particles.

134 **Importance of the P2 and P3 residues of fusion proteins of respiratory viruses for their proteolytic activation by TMPRSS2**

Maino Tahara1, Masako Abe1, Kouji Sakai1, Kazuya Shirato1, Kazuhiko Kanou1, Shutoku Matsuyama1, Hideo Fukuhara2, Katsumi Maenaka2, Yasushi Ami1, Mariko Esumi3, Atsushi Kato1, Makoto Takeda1
1National Institute of Infectious Diseases, Tokyo, Japan,
2Hokkaido University, Hokkaido, Japan
3Nihon University School of Medicine, Tokyo, Japan

We have previously demonstrated that the type II transmembrane serine protease, TMPRSS2, proteolytically activates membrane fusion proteins of human metapneumovirus, human parainfluenza viruses, Sendai virus (SeV), influenza virus, and coronaviruses. In the present study, molecular basis for activation by TMPRSS2 was further analyzed. SeV and influenza virus hardly propagated in Huh7 cells in the absence of trypsin, while they efficiently underwent multicycle infection in TMPRSS2-expressing Huh7 cells. They also failed to undergo multicycle replication in Huh7 cells expressing an enzymatically inactive form of TMPRSS2. Membrane fusion proteins of various respiratory viruses, including SeV, possessed glutamine, serine, and arginine residues (QSR) at positions, P3, P2 and P1, respectively. The QSR residues were identical to those of the self-cleavage site of TMPRSS2. SeVs possessing a mutation at P2 or P3 position (Q114A, Q114S, Q114V, S115R and S115V) were generated, and replication capacity was then analyzed in the null- or TMPRSS2-expressing Huh7 cells. All mutant SeVs underwent multicycle replication using TMPRSS2, but their replication capacity was significantly compromised by mutations, suggesting that SeV F protein possesses residues optimal for proteolytic activation by TMPRSS2. Influenza virus H1, H2, and H3 subtypes possess the same (QSR) or similar (QTR and ESR) motif at the cleavage site. Mutations at P2 and P3 residues of influenza virus HA protein also affect the replication capacity in human respiratory epithelial cells endogenously expressing TMPRSS2. The P2 and P3 residues of viral membrane fusion proteins may play an important role in determining pathology of respiratory viruses.

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

Ayllon Juan 1,4, Hale Benjamin G. 2, Sánchez-Aparicio María T. 1,4, García-Sastre Adolfo 1,3,4
1Department of Microbiology, 3Department of Medicine and 4Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, New York, NY 10029, USA. 2MRC-University of Glasgow Centre for Virus Research, 8 Church Street, Glasgow, G11 5JR, 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 p85α or p85β) and a catalytic subunit, p110, with three isotypes designated α, β and δ. NS1 is known to specifically bind and repress the inhibition caused by p85β. 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.

136 **Modulatory effect of heat shock protein 70 (Hsp70) on influenza A virus polymerase activity**

Rashid Manzoor(1), Kazumichi Kuroda(2), Reiko Yoshida(1), Yoshimi Tsuda(1), Daisuke Fujikura(3), Hiroko Miyamoto(1), Masahiro Kajihara(1), Hiroshi Kida(4), Ayato Takada(1, 5) *Division of Global Epidemiology, Research Center for Zoonosis Control, Hokkaido University, Sapporo, 001-0020, Japan (1); Division of Microbiology, Department of Pathology and Microbiology, Nihon University School of Medicine, Tokyo, 173-8610, Japan (2) ;Division of Infection and Immunity, Research Center for Zoonosis Control, Hokkaido University, Sapporo, 001-0020, Japan (3); Laboratory of Microbiology, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, 060-0818, Japan (4), School of Veterinary Medicine, the University of Zambia, P.O. Box 32379, Lusaka, Zambia (5)*

The role of heat shock protein 70 (Hsp70) in virus replication has been discussed for many viruses. Previous studies have shown that Hsp70 suppresses the influenza virus replication. However, these studies were conducted in cell lines varying in their basal levels of Hsp70. In this study, we determined the role of Hsp70 in influenza virus replication in HeLa and HEK293T cells, which express Hsp70 constitutively and are commonly employed in influenza research. Hsp70 was coprecipitated with PB2, PB1 monomers and PB2-PB1 dimers but not with PA monomers or PB2-PB1-PA trimers. Immunofluorescence microscopy revealed that Hsp70, mainly present in the cytosol, co-localized with PB2 in nuclei of the cells. Knocking down Hsp70 resulted in reduced virus transcription and replication activities. Significantly higher levels of viral polymerase-encoded cRNA and vRNAs were observed during the heat shock phase than recovery phase; suggesting that Hsp70 acted as chaperone for influenza virus polymerase proteins resulting in increased replication activity during heat shock phase when Hsp70 localized in the nucleus where influenza virus replication takes place, but not in the recovery phase when Hsp70 relocated into the cytoplasm. Our data, for the first time, suggest that Hsp70 modulates the influenza virus replication and its previously reported suppressive role could be a sequel of involvement of different cellular pathways.

137 **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 (1), Gülsah Gabriel (1)
1) Heinrich-Pette-Institute, Leibniz Institute for Experimental Virology, Hamburg, Germany
2)University Hospital Hamburg-Eppendorf, Department of Obstetrics and Fetal Medicine, Hamburg, Germany

Differential use of importin-α isoforms governs host adaptation and pathogenesis of influenza viruses in the mammalian host. While growth of highly pathogenic avian influenza viruses depends on importin-α3, growth of mammalian viruses depends on importin-α7 expression in human lung cell cultures. Here, we have compared viral replication efficiency and cell tropism in the lungs of wildtype (WT) and importin-α7-knockout (α7-/-) mice using a recombinant H1N1 influenza virus which carries a GFP reporter gene. We observed that upon GFP-virus infection, 80% of the WT mice succumbed to infection while all α7-/- mice survived. Lungs of WT animals were severely destructed and bronchial and alveolar epithelium was preferentially infected. In contrast, lungs of α7-/- animals were mostly intact with bronchial but not alveolar epithelium infection. Histological studies displayed virus-RNA positive macrophages in the lungs of infected WT but not α7-/- mice. In contrast, no significant differences were detected in virus clearance by primary WT or α7-/- macrophages *in vitro*. The quantification of the cellular immune response in the lungs of infected WT and α7-/- mice by flow cytometry revealed no major differences suggesting that the importin-α7 gene does not modulate cellular immune response pathways. However, higher levels of pro-inflammatory cytokines were detected in the lungs of WT than α7-/- mice potentially contributing to the severe lung pathology observed in WT mice. In summary, our findings show that the importin-α7 gene plays a crucial role in virus replication in the alveolar epithelium and the expression of pro-inflammatory cytokines in the mammalian airway.

138 **Characterization of influenza virus receptors in the respiratory tract of ferrets**

Miranda de Graaf 1, Angela van Diepen 3, Monique I. Spronken 1, Sander Herfst 1, Corneel H. Smit 3, Albert D.M.E. Osterhaus 1, Derek J. Smith 2, Corneel H. Hokke 3, Ron A.M. Fouchier 1
1 Dept of Virology, Erasmus MC, Rotterdam, The Netherlands, 2 Dept of Zoology, University of Cambridge, Cambridge, United Kingdom, 3 Dept of Parasitology, Leiden Medical Center, Leiden, The Netherlands

Influenza viruses cause acute viral disease of the respiratory tract in a wide range of species. The influenza virus hemagglutinin mediates cell entry by binding to sialic acid (SA)-receptors on the host cell-surface. A wide variety of SA-receptors exist in nature, which are species and tissue specific. Human influenza viruses bind to α2,6-Gal terminated SA-receptors, whereas avian influenza viruses prefer those terminating in SA-α2,3-Gal. These gross differences in receptor-binding properties are important determinants of virus host range. However, using glycan array technologies, it has been demonstrated that influenza virus receptor specificity also involves structural modifications of the SA and overall glycan, such as fucosylation, acetylation, and sulphation. Thus to fully understand influenza virus host range and tropism, it is important to understand SA-receptor expression in host tissues. Ferrets are widely used as an animal model for influenza virus pathogenesis. Therefore, the objective of this study was to characterize the glycan composition of the ferret respiratory tract. N-glycans were isolated from nasal turbinates, trachea, bronchus and lungs of ferrets. The N-glycans were released, separated, quantified, and subjected analysis by MALDI-TOF MS(/MS). Digestion with sialidases indicated the presence of both α2,3- and α2,6-linked SA. Only one type of SA, N-acetylneuraminic acid, was detected. This spectrum of glycans can now be compared to those of the human respiratory tract and to glycans present on glycan arrays. Understanding which SA-receptors are present in relevant hosts and tissues will aid the analyses of glycan array data and the identification of determinants of virus host range.

139 **Novel host factors that promote influenza A virus uncoating**

Yohei Yamauchi, Ari Helenius
ETH Zurich

Influenza A virus (IAV) is an enveloped animal virus of the Orthomyxoviridae family with a segmented, negative-sense RNA genome. Once the virus is endocytosed, fusion is mediated by the hemagglutinin (HA) glycoprotein in the acidic environment of late endosomes (LEs) or endolysosomes. This is followed by uncoating of the viral core, and the vRNPs are imported into the nucleus by importin α/β using the classical nuclear import pathway. Here we show that the beta karyopherins TNPO1 and 2 promote efficient IAV uncoating post-fusion and prior to import of vRNPs. RNAi-mediated depletion of TNPO1/2 in A549 cells decreased influenza A/Aichi/68 X31 infection by 95% compared to the control. In cells overexpressing wild-type or dominant-negative TNPO1, infection increased by 30% or decreased by 50%, respectively. We first identified a block in IAV uptake by 75% and a retarded recycling of transferrin. The effect was not totally abolished when the virus was allowed to fuse at the plasma membrane indicating a post-fusion defect. Following acid-bypass at the plasma membrane in TNPO1/2-depleted cells, uncoating of M1 and vRNPs was reduced by 50% and 30%, respectively, compared to the control. Fusion occurred normally. Depletion of importin β, on the other hand, had no effect on M1 or vRNP uncoating but blocked vRNP nuclear import almost completely. These findings suggest that TNPO1/2 promotes IAV uncoating at a post-fusion step before importin β comes into play. Our findings suggest that IAV uncoating is a multi-step process that is initiated by fusion and completed by cytosolic host factors.

140 **Identification of host factors interacting Borna disease virus ribonucleoprotein in the nucleus**

Tomoyuki Honda, Akiko Makino, Kan Fujino, Kozue Sofuku, Shoko Nakamura, Keizo Tomonaga *Department of Viral Oncology, Institute for Virus Research, Kyoto University*

Borna disease virus (BDV), a nonsegmented, negative-strand RNA virus, is characterized highly neurotropic and noncytopathic infection. BDV has several unique features. The most striking feature of BDV is that it establishes a long-lasting persistent infection in the cell nucleus without overt cytopathic effects. This characteristic makes BDV the only animal RNA virus capable of intranuclear parasitism. Therefore, the study of BDV allows us to uncover previously unknown interactions between RNA virus and host factors. Recently, we demonstrated that BDV ribonucleoprotein (RNP) interacts directly with a host chromatin-binding protein, high mobility group box protein 1 (HMGB1), which influences BDV replication and persistent infection. To further investigate the role of HMGB1 in BDV persistence, we isolated HMGB1-binding proteins (HBPs) from the nucleus of BDV-infected cells. We identified that HBP-1, one of HBPs, associated with both HMGB1 and BDV RNP in the nucleus. Knockdown of HBP-1 enhanced BDV replication, suggesting that HBP-1 represses BDV replication. Furthermore, we demonstrated that knockdown of HBP-1 decreased the formation of BDV RNP speckles in the cytosol. These results suggest that HBP-1 might translocate BDV RNP into the cytosol, resulting in the repression of BDV replication. Our data may provide a novel mechanism for regulation of RNA virus replication in the nucleus.

141 **Analysis of cell entry of a novel arenavirus, Lujo virus, using pseudotype VSV**

Hideki Tani1, Masayuki Shimojima1, Shuetsu Fukushi1, Tomoki Yoshikawa1, Masayuki Saijo1, and Shigeru Morikawa2
1. Department of Virology I, 2. Department of Veterinary Science, National Institute of Infectious Diseases, Tokyo, Japan.

Arenaviruses are a major cause of hemorrhagic fevers endemic to Africa and South America, and thus a major public health and medical concern. Because these viruses are categorized as biosafety level 4 pathogens, which are restricted to their use, biological studies including therapeutic drug or vaccine development have been impeded. Here, we have developed pseudotype VSV bearing envelope protein of various species of arenaviruses (AREpv), including recently identified Lujo virus (LUJpv). LUJpv generated in 293T cells exhibited high infectivity in various mammalian cell lines except for mouse derived cell lines. A pH-dependent endocytosis of LUJpv was confirmed by the use of lysosomotropic agents. The infections of New World and Old World AREpv were mediated by human TfR1 and αDG, respectively, while that of LUJpv was not mediated by human TfR1 nor αDG. A pH-dependent endocytosis of AREpv including LUJpv was confirmed by the use of lysosomotropic agents. Cell fusion was induced when the cells expressing envelope proteins of arenavirus, except for Lujo virus, were transiently exposed at low pH. Exposure of LUJpv as well as other AREpv to low pH diminished the infectivity. These results indicate that Lujo virus uses as-yet-unknown receptors for the infection and envelope protein of Lujo virus changes its conformation at low pH in the entry process.

142 **Role of cholesterol transporter proteins NPC1 and NPC1L1 in Lujo virus entry and replication.**

Punya Shrivastava-Ranjan, Ayan K Chakraborti, Stuart T Nichol, Chrsitina F Spiropoulou *Viral Special Pathogens Branch, Division of High Consequence Pathogens and Pathology, Centers for Disease Control and Prevention, Atlanta, GA 30333.*

Lujo virus (LUJV) is a recently identified rodent-borne arenavirus that causes fatal viral hemorrhagic fevers in humans. No specific drugs exist to treat LUJV-caused hemorrhagic fevers. The virus–endosome membrane fusion triggered by the glycoprotein of LUJV is an important step in virus entry, hence potential target for antiviral interventions. The G1glycoprotein of LUJV is highly diverse and distinct from both Old and New Worlds arenaviruses lineage. Niemann Pick C1 (NPC1), a mammalian endosomal cholesterol transporter protein was recently identified as a critical entry factor for filoviruses, though it had no effect on an Old World arenavirus Lassa virus entry. Here we report that NPC1 is required for the cellular entry and replication of infectious LUJV. Using immunofluorescence microscopy we demonstrated that infectious LUJV did not infect NPC1-null primary human fibroblasts derived from Niemann-Pick type C1 disease patients. Furthermore, replication and release of infectious LUJV was significantly decreased in NPC1-null fibroblast in comparison to cells from healthy individuals. Interestingly, we also found that Niemann-Pick C1-like1 protein (NPC1L1), a closely NPC1 related cholesterol transporter protein known to contribute to entry of hepatitis C virus, is also required for the entry of LUJV. Specifically, pre-treating Vero-E6 cells with NPC1L1 inhibitor significantly reduced LUJV replication as seen by immunofluorescence. In contrast, replication of infectious Zaire ebolavirus was not affected by this inhibitor. Our results indicate that both NPC1 and NPC1l are host factors required for LUJV entry and may be potential target for therapeutic intervention.
Lujo virus (LUJV) is a recently identified rodent-borne arenavirus that causes fatal viral hemorrhagic fevers in humans. No specific drugs exist to treat LUJV-caused hemorrhagic fevers. The virus–endosome membrane fusion triggered by the glycoprotein of LUJV is an important step in virus entry, hence potential target for antiviral interventions. The G1glycoprotein of LUJV is highly diverse and distinct from both Old and New Worlds arenaviruses lineage. Niemann Pick C1 (NPC1), a mammalian endosomal cholesterol transporter protein was recently identified as a critical entry factor for filoviruses, though it had no effect on an Old World arenavirus Lassa virus entry. Here we report that NPC1 is required for the cellular entry and replication of infectious LUJV. Using immunofluorescence microscopy we demonstrated that infectious LUJV did not infect NPC1-null primary human fibroblasts derived from NPC1 disease patients. Furthermore, replication and release of infectious LUJV was significantly decreased in NPC1-null fibroblast in comparison to cells from healthy individuals. Interestingly, we also found that NPC1-like1 protein (NPC1-L1), a closely NPC1 related cholesterol transporter protein known to contribute to entry of hepatitis C virus, is also required for the entry of LUJV. Specifically, pre-treating Vero-E6 cells with NPC1L1 inhibitor significantly reduced LUJV replication as seen by immunofluorescence. In contrast, replication of infectious Zaire ebolavirus was not affected by this inhibitor. Our results indicate that both NPC1 and NPC1-L1 are host factors required for LUJV entry and may be potential target for therapeutic intervention.

143 **Cellular adaptation for establishment of measles virus persistent infection in culture**

TOMOMITSU DOI, HIROKI SATO, MISAKO YONEDA, CHIEKO KAI
Laboratory Animal Research Center and International research Center for Infectious Diseases, Institute of Medical Science, University of Tokyo

Measles virus (MV) usually causes cytolytic infection. However, MV also causes persistent infection in specific conditions so that subacute sclerosing panencephalitis is known to develop after the long period persistent infection. To investigate the mechanisms of persistent infection in cultured cells, we previously established a numbers of persistently infected cell lines of MVs. Analysis of features and genome sequence of persistent MVs, we found various mutations on their genome, which are implicated in loss of cytolytic infection. In this study, we focused on the cell features during establishment of MV persistent infection. Virus infection induces cell death associated with syncitium formation until 5 days. After the period, syncitium was not formed and we found that SLAM on the infected cell surface was downregulated. From 5 days to 4 weeks, cell number was hardly increased that was probably caused by plenty of single cell death. Then, cells started to grow normally as parental cells, while viral growth rate was also increased over time during establishment of persistent infection. Sequence analysis of the persistently infected MV genome showed no alteration comparing with parental one. These results suggest that MV persistent infection in culture is also achieved by cellular adaptation without selection of MV subtypes. Cell death caused by MV infection is induced by at least two different mechanisms, syncitium dependent and independent way. Our findings provide a good model to investigate cellular factors involved in interaction between virus and host cells in acute and persistent phase of infection.

144 **DDX1 and DDX3 as potential host factors affecting influenza virus replication in Swine Respiratory Epithelial cells (SRECs)**

Sathya Narayanan Thulasi Raman, Yan Zhou
Vaccine and Infectious Disease Organization, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 5E3, Canada

The NS1 protein of influenza is an important virulence factor and is also multifunctional in nature. This is made possible by its interaction with host and viral proteins in the infected cell. Therefore, it is important to study and understand the physiological role of these interactions. As a platform to study these interactions, we rescued a recombinant swine influenza virus, which could express a strep-tag fused NS1 protein upon infection. The rescued recombinant virus has growth properties and NS1 localization pattern similar to that of the wild type in MDCK cells and the strep-tag NS1 protein is able to bind specifically with high affinity to the strep-tactin resin, thereby facilitating isolation of intact NS1-protein complexes. One of the primary sites of influenza infection in pigs and many other hosts are the tracheal epithelial cells. Hence, studying virus infection in the primary SRECs' would closely resemble that of natural infection. So, we isolated epithelial cells from the trachea of pigs and infected them with the recombinant virus. The strep-tag NS1 protein along with the interacting protein complex was then purified from the infected lysate using the strep-tactin system. The host proteins present in the complex were then identified by mass spectrometry. Several RNA helicases like DDX3, DDX1 and DDX17 were identified along with many other proteins present in the complex. Since, influenza is an RNA virus, we hypothesized that these proteins might be involved in the viral life cycle.

145 **Influenza A virus protein PB1-F2 reduces viral pathogenesis in chicken**

Olivier Leymarie 1, Hana Weingartl 2, Bernard Delmas 1,Ronan Le Goffic 1
1 INRA 78350 Jouy-en-Josas
2 Special Pathogens Unit, National Centre for Foreign Animal Disease, Canadian Food Inspection Agency, Winnipeg, Manitoba, Canada.

Since 1997, H5N1 avian influenza viruses (AIV) have caused numerous outbreaks in poultry. Waterfowl and shorebirds constitute the natural host of H5N1 AIV, in which infection is usually asymptomatic or results in limited disease. Nevertheless, when the virus is transmitted to poultry, mortality rate can reach 100%. Among the viral effectors that modulate virulence in these species, the PB1-F2 protein was shown to contribute to viral pathogenesis but its function in avian host is not well understood. In this work, we compared the effect of a wild-type (wt) highly virulent H5N1 AIV strain with its mutant unable to express PB1-F2 (ΔF2) during chicken infection. Unexpectedly, we first observed that wt-infected chickens had a higher survival rate than the ΔF2-infected chickens, suggesting that the expression of PB1-F2 during AIV infection could be beneficial for the avian host. Remarkably, the biodistribution analysis of the two viruses revealed a higher viral titer of the wt virus within intestine, indicating that PB1-F2 has an impact on the spreading of the virus. Finally, we compared the host response of the chickens to the two viruses within lungs and in blood cells. In the lung, PB1-F2 expression results in a potent down-regulation of the immune response. In blood cells, a gene signature associated to mitochondrial dysfunction was observed. Collectively, our data suggest that PB1-F2 expression deeply affect the immune host response in chickens in a way that attenuates pathogenicity, a feature that contrast with what was previously observed in mammal species.

146 **Characterization of the role of human cathepsin W in the entry process of influenza A virus**

Thomas Edinger, Marie-Theres Pohl, Silke Stertz
University of Zurich Institute of Medical Virology
Winterthurerstrasse 190 .8057 Zürich Switzerland

For the entry process of influenza A virus (IAV) a broad range of host factors is required. This is due to the fact that IAV has only a small coding capacity with its (-)ssRNA genome and for the entry and following replication it is dependent on a repertoire of host cell proteins. By performing genome wide siRNA screens several of these host factors were identified. We have tested a number of the identified factors in a VLP-based entry assay and found several host proteins that are required for entry. One of the hits is the lysosomal peptidase, human cathepsin W (CtsW). The VLP-based entry assay in siCtsW treated cells showed a reduction for influenza VLP entry but not for VLPs harbouring viral glycoproteins of other viral species. Furthermore siCtsW treatement of A549 lung epithelial cells and subsequent infection resulted in a reduced viral titer for influenza A virus A/WSN/33. By applying confocal microscopy we observed a reduction in incoming vRNPs in the nuclei of siCtsW treated cells and vRNPs are accumulating in the cytoplasm after three hours of infection, instead of entering the nucleus. When we stained for the early endsomal marker EEA1, we found equal co-localization levels of EEA1 and NP in siCtsW treated cells and control cells. This leads to the assumption that a late step of the entry process is affected. We are currently using several cell-biological and molecular methods to get a more detailed understanding of how this host factor is involved in the viral entry process.

147 **Primary airway epithelial cells from swine provide a model system to study proteolytic activation of influenza virases**

C. Peitsch, H.- D. Klenk, W. Garten, E. Böttcher-Friebertshäuser
Institute of Virology, Philipps University Marburg, Hans-Meerwein-Straße 2, 35043 Marburg, Germany

Influenza A viruses cause an acute respiratory disease that affects millions of people during seasonal epidemics and occasional pandemics. Due to their natural susceptibility to avian and human influenza viruses, swine may serve as intermediate hosts and play an important role in the epidemiology of influenza A viruses. As in humans, respiratory epithelial cells are the primary target cells for influenza viruses in swine. Cleavage of the viral surface glycoprotein hemagglutinin (HA) by host cell proteases is essential for viral infectivity. Hence, it can be assumed that the very same HA-activating mechanism is accomplished by porcine proteases homologous or similar to human HA-processing proteases. We analysed proteolytic activation of influenza virus HA in primary swine airway epithelial cells. Seasonal and pandemic influenza A viruses replicated efficiently in the epithelial cells in the absence of exogenous HA-activating proteases. Interestingly, swine proteases swTMPRSS2 and swAT, homologues to human HA-activating proteases TMPRSS2 and HAT, were found to be expressed in several sections of the porcine respiratory tract. We cloned swTMPRSS2 and swAT from primary bronchial epithelial cells and demonstrated that both are able to cleave HA and support proteolytic activation of influenza A viruses. Consistent with the human homologues, expression of swTMPRSS2 and swAT and, most likely, HA cleavage differ in subcellular localisation. In conclusion, swTMPRSS2 and swAT are promising candidates for proteolytic activation of influenza virus HA in the respiratory tract of pigs and, moreover, primary swine airway epithelial cells constitute a suitable in vitro model system for influenza A virus infections.

148 **The regulation and functional significance of GALNT3 expression during influenza A virus infection**

Shoko Nakamura, Masayuki Horie, Mayo Yasugi, Daisuke Okuzaki, Akiko Makino, Tomoyuki Honda, Keizo Tomonaga
1. Department of Viral Oncology, Institute for Virus Research, Kyoto University, Japan 2. Graduate School of Life and Environmental Sciences, Osaka Prefecture University 3. Department of Molecular Genetics, Research Institute for Microbial Disease (BIKEN), Osaka University

Viral infections affecting the upper or lower respiratory tract induce the production of mucus in the epithelial surfaces of the respiratory cells. The mucus is largely formed by mucins, which are complex O-linked glycosylated proteins. Recent studies revealed that the expression of mucins dramatically increases in the respiratory epithelial cells after some virus infections. However, a little is known about how mucins are produced on the surfaces of respiratory epithelial cells and involved in viral infections. Influenza A virus (IAV) causes the acute upper respiratory infection. In this study, we identified that two miRNAs, which could regulate the expression of GalNAc transferase 3 (GALNT3), rapidly increase at the early phase of IAV infection and may be involved in the process of mucin production. At first, to understand the regulatory mechanism of GALNT3 expression by identified miRNAs, we analyzed the binding of miRNAs to the 3' UTR of GALNT3 mRNA. Furthermore, we investigated the relationship between GALNT3 expression and IAV strain A/Puerto Rico/8/34 (PR8) replication in the upper respiratory epithelial cells. As a result of luciferase reporter assay analysis, two miRNAs actually interacted with 3' UTR of GALNT3 mRNA and regulated GALNT3 expression. Analysis using siRNA against GALNT3 revealed that upregulation of GALNT3 during IAV infection may be associated with IAV gene transcription and replication. We are currently investigating in detail whether the expression of GALNT3 directly associates with IAV replication and how O-glycosylation of mucins and other substrates of GALNT3 act against IAV infection in infected cells.

149 **Nairobi sheep disease virus causes translocation of ER chaperones in infected cells**

Lidia Lasecka1, Haru-Hisa Takamatsu1, Dirk Werling2 Michael D Baron1
1The Pirbright Institute, Pirbright, Surrey, UK
2Royal Veterinary College, Hatfield, Herts, UK

Nairobi sheep disease virus (NSDV) of the genus Nairovirus causes a haemorrhagic gastroenteritis in sheep and goats with mortality up to 90%. The virus is closely related to the human pathogen Crimean-Congo haemorrhagic fever virus (CCHFV). Replication of NSDV takes place in the cytoplasm and the new virus particles bud into the Golgi apparatus; however, the effect of replication on cellular compartments has not been studied extensively. Protein disulphide isomerase (PDI) is a chaperone, present in lumen of endoplasmic reticulum (ER), which assists during protein folding. Using confocal microscopy, we observed that NSDV infection led to the loss of PDI from the ER. However, the structure of the ER, as judged by the distribution of the ER membrane protein calnexin, was not affected. Further investigation showed that NSDV-infected cells have high levels of PDI at their surface, and the protein is also secreted into the culture medium above infected cells. Another chaperone from the PDI family, ERp57, was found to be similarly affected. Expression of individual viral glycoproteins showed that expression of Gn led to similar loss of PDI from the ER. PDI can be secreted from and detected at the surface of activated platelets and endothelial cells, and it has been suggested that this PDI can activate tissue factor and integrins, involved in coagulation and inflammation respectively. The discovery of enhanced PDI secretion from NSDV-infected cells may be a crucial finding for understanding the mechanisms underlying the pathogenicity of nairoviruses.

150 **Junin virus NP but not Tacaribe virus NP serves a decoy-function to prevent apoptosis induction during infection**

Svenja Wolff (1), Allison Groseth (1,2), Stephan Becker (1)
(1) Institute of Virology, Philipps-University Marburg, Germany
(2) Laboratory of Virology, DIR, NIAID, NIH, Hamilton, MT, USA

The regulation of apoptosis during infection is an important factor for host survival and, in some cases, also for the virus life cycle. It is, therefore, interesting to understand how viruses interfere with the cellular apoptosis machinery. However, the role of apoptosis during arenavirus infection is enigmatic. Junin virus (JUNV) is the causative agent of Argentine hemorrhagic fever and belongs, like its apathogenic relative Tacaribe virus (TCRV), to the New World arenaviruses. Our study was initiated by the finding that during JUNV infection, as well as recombinant expression, the JUNV nucleoprotein (NP) is cleaved into discrete products. TCRV NP, however, does not show a comparable specific degradation pattern. Examination of the JUNV NP sequence revealed several putative caspase cleavage motifs. Point mutations of these motifs led to alterations in the observed degradation pattern and were consistent with loss of certain cleavage events. Since caspase activity also influenced NP cleavage in transfected cells, we propose JUNV NP as a substrate of caspases. We further could show that expression of JUNV NP alone, but not of TCRV NP, suppresses the induction of apoptosis in cells treated with an apoptosis activator and that this anti-apoptotic effect depends on cleavage of JUNV NP. Taken together, our data reveal that JUNV NP, in contrast to TCRV NP, serves as a target for caspase cleavage and is, therefore, able to prevent the induction of apoptosis in JUNV infected cells, possibly as by serving as a decoy substrate.

151 **Prolidase (PEPD) is a required human factor for influenza A virus entry**

Marie-Theres Pohl, Thomas Edinger, Silke Stertz
Institute of Medical Virology, University of Zurich, 8057 Zurich, Switzerland

Influenza A virus causes an acute respiratory illness and is a constant threat to public health. The identification of human factors that favor viral propagation may promote the development of novel antiviral drugs against influenza A virus (IAV). Recently, five genome-wide siRNA screens have been performed to determine host factors of IAV infection but surprisingly, there was little overlap between primary hits of those screens. We revisited the primary hits of these screens to determine and characterize novel host factors that are associated with the entry process of IAV. We focused on 44 primary hits lacking experimental validation, which are common of two or more screens. Using a VLP-based entry assay we identified 23 potential candidates to be involved in IAV entry. One of the well-performing candidates is prolidase (PEPD), a ubiquitously expressed cytosolic enzyme that hydrolyzes dipeptides with proline or hydroxyproline at the C-terminus. Further validation experiments revealed that siRNA-mediated knockdown of PEPD reduces wild-type IAV single- as well as multi-cycle growth. This effect was observed not only for the lab-adapted strain A/WSN/33 but also for the two seasonal IAV strains A/HK/68 (H3N2) and A/Texas/36/91 (H1N1). In addition, we monitored IAV entry by confocal microscopy in siRNA-treated cells. Control cells showed robust viral protein expression in the nucleus 3 hours post-infection. In PEPD knockdown cells however, nuclear staining of viral proteins was largely absent indicating a defect early in the viral life cycle. Overall, our studies establish PEPD as a human host factor required for efficient IAV entry.

152 **Host-dependence of the thermosensibility of measles virus vaccine strains and critical function of HSP90 in viral replication**

Louis-Marie Bloyet, Jérémy Welsch, Jessica Rabilloud, Branka Horvat, Denis Gerlier, Boyan Grigorov
CIRI, INSERM U1111, CNRS, Université Lyon 1, ENS Lyon, CERVI, 21 Avenue Tony Garnier 69007 Lyon.

Attenuation of measles virus (MeV) that gave rise to a well-tolerated vaccine was ultimately reached after adaptation to grow into chicken embryonic fibroblasts (CEF). Moraten and Schwarz vaccines have been selected for their ability to grow in CEF at 32°C but not at 37°C. This thermosensibility is not intrinsic to the viruses as they displayed a normal growth at 37 °C in human or simian cells. A correlation between the temperature-restriction of viral growth and a decreased expression of HSP90 in CEF lead us to investigate the role of HSP90 in MeV replication cycle. In these cells, HSP90 appears to be a limiting factor since overexpression of HSP90 in CEF enhanced MeV growth. Furthermore, inhibition of HSP90 either by siRNA or by addition of a chemical inhibitor decreased or abrogated the viral RNA synthesis and viral production not only in CEF but also in other cell types such as Vero cells. Kinetics experiments and reversibility of the inhibition upon drug retrieval pointed to the polymerase complex as a likely target of HSP90 activity. Upon blocking HSP90, the amount of MeV L protein detectable by western blot was strongly decreased, while P expression remained unaffected. Treatment of the cells with a proteasome inhibitor did not prevent the decrease induced by the HSP90 inhibitor. HSP90 was detected in viral particles suggesting a direct interaction between L and HSP90 and ongoing experiments aim at investigating this interaction.

153 **Novel insights into the divergent mechanisms of paramyxovirus immune evasion**

Michelle Audsley (1), Glenn Marsh (2), Mary Tachedjian (2), Linfa Wang (2), David Jans (1), and Gregory Moseley (1).
(1) Department of Biochemistry and Molecular Biology, Monash University
(2) Australian Animal Health Laboratories, CSIRO

The Paramyxovirus family includes a number of viruses that pose significant global health threats to humans and livestock, including measles virus (>150,000 human deaths/annum) and zoonotic bat-borne viruses of the henipavirus genus (Nipah and Hendra) that have invaded human populations with case-fatality rates as high as 70%. Their capacity to evade interferon (IFN)-mediated innate immune responses is a major determinant of pathogenicity and host specificity, and is dependent on products of the viral P-gene, including the V-protein. V-protein antagonism of IFN-signalling through mechanisms including proteasomal degradation has represented an archetype in viral immune evasion. However, it is becoming increasingly clear that immune evasion mechanisms are highly diverse and only partially understood among the >30 paramyxoviruses, with many paramyxoviruses entirely uncharacterised in this respect. Using an approach combining functional genomic analysis of infected cells, live-cell imaging, and IFN-signalling assays, we have examined the xpression/functions of P-gene-encoded proteins from a panel of emerging zoonotic paramyxoviruses, including members of the lethal henipavirus genus. We found that although almost all paramyxovirus V-proteins bind the immune signalling factors STAT1/2 and MDA5, members of a potential new genus isolated from rodent/bat hosts show significant divergence in this regard. We also characterised unique interactions of specific P-gene-encoded proteins with the cellular cytoskeleton and components of the nuclear transport machinery, suggesting they may be involved in novel immune evasion strategies. Furthermore, recent results suggest pharmacological inhibition of these interactions can reduce infection by henipaviruses, and our current research is focused on validating these interactions as new therapeutic targets.

154 **Paramyxovirus V proteins disrupt the fold of the innate immune sensor MDA5 to inhibit antiviral interferon response**

Carina Motz (1), Kerstin Monika Schuhmann (1,2), Axel Kirchhofer (1,3), Manuela Moldt (1), Gregor Witte (1), Karl-Klaus Conzelmann (1,2), Karl-Peter Hopfner (1)
(1) Gene Center Munich, LMU
(2) Max von Pettenkofer-Institute, LMU Munich
(3) Business Consulting, Bayer Business Services GmbH, Leverkusen

RIG-I like receptors (RLR: RIG-I, MDA5 and LGP2) of the innate immune system sense cytoplasmic viral RNA and trigger signaling cascades that lead to an antiviral state of the cell. However, viruses employ different strategies to counteract RLR signaling and dampen the host interferon response. While we begin to understand how RLRs mechanistically sense viral RNA, molecular mechanisms of viral interference with RLRs remain unclear. Paramyxoviruses inactivate MDA5 with their V protein. We report the crystal structure of a complex between parainfluenza virus 5 (PIV5) V protein and MDA5's ATP hydrolysis domain that explains how V proteins inhibit MDA5. Remarkably, V protein unfolds MDA5's ATPase domain via a ß-hairpin motif and recognizes a structural motif of MDA5 that is normally buried in the conserved helicase fold. The ß-hairpin of V replaces two ß-strands of the MDA5 structural core, disrupts MDA5's ATP-hydrolysis site and prevents formation of nucleic acid bound MDA5 filaments. V protein exposes the ß-hairpin like a spring blade, whereby the remainder of V protein forms a cocoon that prior to MDA5 binding shields the ß-hairpin by mimicking the MDA5 target site. Our data explain how V proteins inactivate MDA5 and LGP2, but not RIG-I. Mutating only two amino acids in RIG-I induces robust V protein binding, validating our results by gain of function. The structure of a viral inhibitor bound to an innate immune receptor uncovers an intriguing mechanism of inhibition of RLR signalosome formation by double unfolding of both receptor and inhibitor.

155 **Paramyxovirus V proteins interact with the TRIM25/RIG-I regulatory complex and inhibit RIG-I signaling**

Leighland J. Feinman: (1/2), Maria T. Sanchez-Aparicio: (1), Adolfo García-Sastre: (1/2/3), Megan L. Shaw (1/2)
Dept of Microbiology (1) Graduate School of Biomedical Sciences (2) Global Health and Emerging Pathogens Institute (3) Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

Like other paramyxovirus V proteins, the Nipah virus (NiV) V protein is a known antagonist of the RIG-I like receptor (RLR)-mediated interferon (IFN) induction pathway, acting through and interacting with the RLR MDA5. It is also known to interact with the RLR LGP2, though the physiological results of this interaction are unclear. We report an interaction between the NiV V protein and the third known RLR, RIG-I. We have also observed interactions between RIG-I and the measles virus and Sendai virus V proteins, but not with the PIV5 V protein. We have determined that for the NiV V protein, interaction with RIG-I depends on the presence of the conserved paramyxovirus V C-terminal region. Furthermore, we have determined that this interaction facilitates the participation of the NiV V protein in the RIG-I/TRIM25 regulatory complex, which results in dysregulation of RIG-I ubiquitination by TRIM25 and disrupts RIG-I signaling. This is a novel mechanism for innate immune inhibition by NiV V, distinct from both STAT1 and MDA5 interaction activities.

156 **Molecular basis of RIG-I activation**

Stéphanie ANCHISI, Jessica GUERRA, Dominique GARCIN
Département de Microbiologie & Médecine Moléculaire - CMU Genève

RIG-I, a cytosolic protein of the RIG-I like receptors family, is known to be activated by diverse positive and negative strand RNA viruses. Some of the molecular means allowing RIG-I to differentiate between cellular and viral RNA have been identified in previous studies: double-stranded RNA with a blunt-end and 5'triphosphate referred to as pathogen-associated molecular patterns ("PAMPs"). The binding of RNA PAMP (on the C-terminal domain of RIG-I) induces conformational changes allowing ATP binding and CARD domains release required to initiate downstream signalling (*i.e.* interferon-β induction). However, the manner in which RIG-I is activated remains unclear. For this purpose, we designed various RNA molecules: single-strand (ssRNA) or double-strand (dsRNA), with a 5' triphosphate (5'ppp) or hydroxyl group (5'OH), as well as chimeric 3' strand hybrids with both ribo- and deoxyribo-nucleotides. These molecules were tested using 3 different experimental approaches: RNA pull-down assays, *in vitro* ATPase and *in vivo* IFNβ induction. Our results suggest that RIG-I is in contact with both strands and interacts with different elements to switch on an activated state: i) For stable binding to RIG-I, a blunt 5'ppp double-strand structure is required. ii) For ATP binding and ATPase activity, further features are involved: contact with ribonucleotides 2 and 5 on the 3' strand. iii) For full RIG-I activation, *i.e.* IFNβ induction, additional ribonucleotides are necessary on the 3' strand (between 10 to 20). Altogether, these results highlight the importance of structural features for RIG-I activation and allow us to define a new model of this molecular mechanism.

157 **Unanchored Lysine48-linked polyubiquitin chains positively regulate the type I IFN-mediated antiviral response**

Ricardo Rajsbaum1,2, Gijs A. Versteeg1,2, Sonja Schmid1, Ana M. Maestre1, Alan Belicha-Villanueva1,2, Jenish R. Patel1, Juliet Morrison1,2, Giuseppe Pisanelli1,2,4, Ana Fernandez-Sesma1,2, Benjamin R. tenOever1,2, Adolfo García-Sastre1,2,3.
1Department of Microbiology, 2Global Health and Emerging Pathogens Institute and 3Department of Medicine, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, New York, NY 10029, USA; 4Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy

Type-I interferons (IFN-I) are essential antiviral cytokines produced upon microbial infection. IFN-I elicits this activity through the upregulation of hundreds of IFN-I stimulated genes (ISGs), many of which have known antiviral activity. The full breadth of ISG induction requires activation of a number of cellular factors including the IκB kinase epsilon (IKKε). However, the mechanism of IKKε activation upon viral infection or IFN receptor signaling remains elusive. Here we show that TRIM6, a member of the E3-ubiquitin ligase tripartite motif (TRIM) family of proteins, interacts with IKKε and promotes induction of IKKε-dependent ISGs. Some studies have suggested a role for unanchored K63-linked polyubiquitin chains, which are not conjugated to any protein, in regulation of kinase activity. However, no role has yet been established for unanchored K48-linked polyubiquitin chains in kinase activation. We show that TRIM6 and the E2-ubiquitin conjugase UbE2K cooperate in the synthesis of unanchored K48-linked polyubiquitin chains, which activate IKKε for subsequent STAT1 phosphorylation. Mechanistically, these unanchored ubiquitin chains promote IKKε oligomerization and autophosphorylation, required for downstream signaling. Our work defines a previously unrecognized activating role of K48-linked unanchored polyubiquitin chains in kinase activation and identifies the UbE2K-TRIM6-ubiquitin axis as critical for IFN signaling and antiviral response.

158 **STAT2 deficiency and susceptibility to (NSV) viral illness in humans**

Sophie Hambleton1,2, Stephen Goodbourn3, Dan F. Young4, Paul Dickinson9, Siti M.B. Mohamad1,5, Manoj Valappil6, Naomi McGovern1, Andrew J. Cant 1,2, Scott Hackett7, Peter Ghazal9, Neil V. Morgan8 & Richard E. Randall4 *1Primary Immunodeficiency Group, Institute of Cellular Medicine, Newcastle University, UK. 2Paediatric Immunology Service, Great North Children's Hospital, Newcastle upon Tyne, UK 3Division of BioMedical Sciences, St George's, University of London, SW17 0RE, UK, 4School of Biology, University of St Andrews, UK,, 5Advanced Medical and Dental Institute, Universiti Sains Malaysia, Penang, Malaysia, 6Health Protection Agency, Royal Victoria Infirmary, Newcastle upon Tyne, UK, 7Paediatric Immunology Dept, Heartlands Hospital, Birmingham, UK,8Institute for Biomedical Research, University of Birmingham, UK, 9Divison of Pathway Medicine, University of Edinburgh, UK*

Severe infectious disease in children may be a manifestation of primary immunodeficiency. These genetic disorders represent important experiments of nature with the capacity to elucidate nonredundant mechanisms of human immunity. We hypothesized that a novel primary defect of innate antiviral immunity was responsible for unusually severe viral illness in two siblings; the proband developed disseminated vaccine strain measles following routine immunization, while an infant brother died after a two-day febrile illness from an unknown viral infection. Patient fibroblasts were indeed abnormally permissive for viral replication in vitro, associated with profound failure of type I interferon signaling and absence of STAT2 protein. Sequencing of genomic DNA and RNA revealed a homozygous mutation in intron 4 of STAT2 that prevented correct splicing in patient cells. Subsequently, other family members were identified with the same genetic lesion. Despite documented infection by known viral pathogens (including influenza viruses, paramyxoviruses and herpesviruses) some of which have been more severe than normal, surviving STAT2-deficient individuals have remained generally healthy, with no obvious defects in their adaptive immunity or developmental abnormalities. These findings imply that type I IFN signaling (through ISGF3) is surprisingly not essential for host defense against the majority of common childhood viral infections. The implications of these findings in terms of our understanding of the importance of type I IFN signaling in controlling negative sense RNA virus infections will be discussed.

159 **Differences in interferon β inhibition between the C proteins of measles virus vaccine and wildtype strains**

Konstantin Sparrer 1, Christian K. Pfaller 2, Karl-Klaus Conzelmann 1
1 Max von Pettenkofer-Institute & GeneCenter, Feodor-Lynen-Str 25, 81377 München
2 University of California at Santa Barbara, Santa Barbara, CA 93106 USA

Effective vaccination relies on the rapid and efficient recognition of a virus by the human innate immune system. Measles virus (MV, Genus Morbillivirus), a member of the paramyxovirus family, is known to induce lifelong immunity against reinfection. However, the acute infection is associated with a dramatic immunosuppression, accompanied by secondary infections, which can eventually kill a host. The pathogenesis of established MV vaccine strains is less severe and the typical immunosuppression is not observed to the same extent, while life-long protection against MV is induced as well, indicating efficient stimulation of the immune system. Infection of cells is sensed predominantly by RIG-I recognizing viral 5'-triphosphate RNAs, and MDA5. We have identified the 186 amino acid MV C protein, which shuttles between the nucleus and cytoplasm, as a major viral inhibitor of IFN-beta transcription in human cells. Notably, C proteins of wildtype MV isolates, being poor IFN-beta inducers, were found to comprise a canonical nuclear localization signal (NLS), whereas the respective sequence of all vaccine strains, irrespective of their origin, was mutated. Site-directed mutagenesis of the C proteins from a MV wildtype isolate and from a vaccine virus confirmed a correlation of nuclear localization and inhibition of IFN-β transcription. A functional NLS and efficient nuclear accumulation is therefore critical for MV C to retain its potential to downregulate IFN-ß induction. Therefore we suggest that a defect in efficient nuclear import of C protein contributes to the attenuation of MV vaccine strains and indicates an important difference between vaccine and wildtype strains.

160 **The matrix protein of rabies virus binds to RelAp43 to suppress NF-κ-B dependent gene expression related to innate immunity**

Youcef Ben Khalifa (1), Sophie Luco (1, 2), Mehdi Archambaud (1, 2), Olivier Delmas *(1), Jonathan Grimes (3), Hervé Bourhy (1)*
(1) Institut Pasteur, Unité Dynamique des Lyssavirus et Adaptation a l'hote, 25 rue du docteur Roux, 75015 Paris, France.
(2) Université Paris Diderot, Sorbonne Paris Cité, Cellule Pasteur, rue du docteur Roux, 75015 Paris, France.
(3) Division of Structural Biology, Welcome Trust Center for human Genetics, University of Oxford, Oxford, United Kingdom

The activation of expression of genes involved in innate immune response is controlled by the activity of transcription factors such as the proteins of the NF-κB family. Recently, we identified a sixth member of this family, RelAp43, which is involved in the activation of INF-β transcription during viral infection (Luco et al., PLoS Pathog 2012). The matrix (M) protein of wild isolates of rabies virus such as Tha (M-Tha) is able to interact with RelAp43 and to efficiently suppress NF-kB dependent reporter gene expression, in response to activation by tumor necrosis factor (TNF), in contrast with the vaccine strain SAD. We then studied the capacity of various truncated and site mutated mutants of M to interact with RelAp43 by co-IP experiments and to activate the NF-κB pathway and identified the region of M and the residues involved in these functions. Moreover, we selected a subset of genes involved in antiviral response and we identified four genes as particularly down regulated by M-Tha compared to M-SAD: CYCLD, NFKB1, STAT1 and TRIM25. Finally, we used an approach that relies on small interfering RNA and overexpression of RelAp43 to study the modulation of genes involved in innate immunity and in NF-κB signaling. Thus, RABV-M protein appears as a potent viral immune-modulatory factor that prevents NF-κB dependent gene expression by fixation of RelAp43.

161 **Sequestration of RNA-regulating proteins in Ebola virus inclusions**

Kristina Maria Schmidt (1,2), Emily Nelson (1,2), Judith Olejnik (1,2), Kristina Brauburger (1,2), Travis Taylor (3), Logan Banadyga (4), Hideki Ebihara (4), Elke Mühlberger (1,2)
(1) Department of Microbiology, Boston University School of Medicine and (2) National Emerging Infectious Diseases Laboratories, Boston, MA; (3) University of Toledo, Department of Medical Microbiology and Immunology, Toledo, OH; (4) Laboratory of Virology, Rocky Mountain Laboratories, DIR, NIAID, NIH, Hamilton, MT

Ebola virus (EBOV) infection causes a severe hemorrhagic fever in humans with case fatality rates up to 90%. EBOV nucleocapsid (NC) proteins form cytoplasmic inclusions, which are sites of viral replication. An important antiviral defense mechanism is stress-induced translational arrest, accompanied by the formation of cytoplasmic stress granules (SGs). These SGs contain stalled translation pre-initiation complexes and RNA-binding proteins, known to stabilize or destabilize sequestered mRNAs. In this study, we analyzed the cellular stress response to EBOV infection. SG formation was not observed in EBOV-infected cells. However, various SG RNA-binding proteins accumulated within the viral inclusions. The host proteins sequestered in the inclusions did not relocate upon induction of SGs using different stress inducers. While ectopic expression of the NC proteins was not sufficient to recruit SG proteins into the inclusions, SG proteins were sequestered in viral inclusions containing active polymerase complexes, indicating that the presence of viral RNA is a prerequisite for SG protein sequestration. In addition, we observed that cellular RNA-binding proteins are involved in the regulation of viral mRNA stability. To further understand how EBOV interacts with SG components, EBOV NC proteins were individually examined for their ability to interact with SGs. Interestingly, the polymerase cofactor VP35, which antagonizes important host antiviral pathways, colocalizes with SGs and inhibits SG formation when expressed at high levels. Our results suggest that the sequestration of SG RNA-binding proteins within the inclusions plays an important role in the viral replication cycle and prevents the formation of SGs in EBOV-infected cells.

162 **Ebola Virus Secreted Glycoprotein Alters the Host Antibody Response via "Antigenic Subversion"**

Gopi S. Mohan, Ling Ye, Chinglai Yang, Richard W. Compans
Department of Microbiology and Immunology, Emory University School of Medicine

In addition to its surface glycoprotein (GP1,2), Ebola virus (EBOV) directs the production of large quantities of a nonstructural secreted glycoprotein (sGP) whose function has been widely debated. The secretion of viral antigens has been studied in several viruses as a mechanism of host immune evasion via competition for virus-specific antibodies. However, such activity has not been conclusively demonstrated for EBOV sGP. Here, we immunized mice with DNA constructs expressing GP1,2 and/or sGP, and demonstrate that sGP can efficiently compete for anti-GP¬1,2 antibodies from mice immunized against sGP, or both sGP + GP¬1,2¬.. However, sGP cannot compete for anti-GP1,2 antibodies from mice immunized solely against GP¬1,2¬. Furthermore, exposure to sGP can induce a previously GP1,2-immunized mouse to adopt cross-reactivity with sGP. We term this phenomenon "antigenic subversion", and propose a model whereby sGP induces the host immune response to focus on epitopes shared between sGP and GP1,2¬, thereby allowing sGP to bind and compete for anti-GP1,2 antibodies. This is distinct from previously proposed "decoy" mechanisms in which secreted antigens passively absorb virus-specific antibodies, since subversion requires exposure to sGP during generation of the anti-GP immune response. While EBOV likely originally evolved this strategy to survive within its chronically infected natural reservoir, antigenic subversion may also represent an important obstacle to EBOV vaccinology. Our findings underscore the importance of rapidly clearing an EBOV infection before antigenic subversion can occur, and further suggest that it may be desirable to design vaccines which lack sGP-reactive epitopes in order to avoid subversion.

163 **Respiratory syncytial virus (RSV) NS1 protein inhibits host cell apoptosis mediated by the interferon induced transmembrane prot**

Shirin Munir, Peter L. Collins
Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA

RSV is the leading viral cause of severe pediatric respiratory infections worldwide. The RSV nonstructural proteins NS1 and NS2 block type I interferon (IFN) induction and signaling and also suppress apoptosis of infected cells, with NS1 having the greater effect in both activities. The mechanism by which apoptosis is inhibited and whether it is related to IFN antagonism remains unclear. Using a yeast-two-hybrid screen, we identified IFITM2 and 3 as NS1-interacting factors. Using cDNA expression and viral infection, we found that: (1) IFITM2 and 3 are RSV restriction factors and significantly reduce RSV replication, (2) IFITM2 and 3 cause cell death that occurs by apoptosis in case of IFITM3, (3) overexpression of NS1 countered IFITM-induced apoptosis, (4) knockdown of IFITM expression in RSV-infected cells reduced apoptosis and increased RSV gene expression, and (5) NS1 binds to the IFITMs during RSV infection and likely inhibits apoptosis by sequestering them. These data suggest that IFITMs play a role in the increased apoptosis observed during infection with RSV lacking NS1. IFITM2 and 3 are expressed constitutively and also are strongly induced by IFN treatment as well as by RSV infection. However, the accumulation of IFITM proteins decreased sharply during RSV infection, and this was found to be due to protein degradation by the 26S proteasome and was independent of NS1. These data suggest that RSV blocks IFITM activity (1) by physically binding and sequestering IFITM by expression of NS1, and (2) by stimulating their degradation in an unknown fashion.

164 **Utilization of host ubiquitin ligases by Rift Valley fever virus virulence factor NSs**

Markus Kainulainen 1, Matthias Habjan 2 3, Laura Busch 1, Philipp Hubel 3, Simone Lau 1, Andreas Pichlmair 3 4, Giulio Superti-Furga 4, Friedemann Weber 1 2
1 Philipps-University Marburg Germany,
2 University of Freiburg Germany,
3 Max Planck Institute of Biochemistry Martinsried Germany,
4 CeMM Vienna Austria

Rift Valley fever virus (RVFV, *Phlebovirus / Bunyaviridae*) is an arbovirus causing large epizootics and epidemics in Africa and on the Arabian Peninsula. In animals, the disease is characterized by high rates of abortions and death of young animals. In humans, symptoms vary from mild self-limiting disease to haemorrhagic fever and death. The main virulence factor of RVFV is the non-structural protein of the small genome segment (NSs). Work by our group and others has shown that NSs is able to limit the cell antiviral response by downregulating host transcription while rescuing translation. These effects are achieved by targeting two host factors, p62 and PKR, for proteasomal degradation (Kalveram et al., J. Virol. 2011, Ikegami et al.,PLoS Pathog. 2009, Habjan et al., J. Virol. 2009). p62 is a component of the general transcription factor TFIIH. PKR is an RNA-activated protein kinase which inhibits virus multiplication by downregulating protein translation. Our group is interested in defining how NSs targets these factors. We have identified a host cell E3 ubiquitin ligase that interacts with NSs. This factor is specifically required for degradation of p62 by NSs, but not for the degradation of PKR. When the ligase is knocked down, p62 is preserved and localizes in the nuclear filaments characteristically formed by NSs. Partial rescue of the antiviral type I interferon response is detected in these cells, showing that the ligase is relevant regarding the virulence mechanism of NSs. We are currently screening for factors required for the NSs induced PKR degradation.

165 **Interferon-inducible Mx1 protein inhibits influenza virus by interfering with viral ribonucleoprotein complex assembly**

Judith Verhelst (1,2), Eef Parthoens (1,2), Bert Schepens (1,2), and Xavier Saelens (1,2)
(1) Department for Molecular Biomedical Research, VIB, 9052 Ghent, Belgium
(2) Department of Biomedical Molecular Biology, Ghent University, 9052 Ghent, Belgium

Mx proteins are large GTPases that are induced by type I and type III IFNs. These proteins are present in most vertebrates and inhibit a wide range of viruses, including many negative stranded RNA viruses. Mouse Mx1 was discovered based on its strong resistance against influenza A virus infection. Later, it was shown that Mx1 inhibits influenza virus infection by blocking viral transcription and replication, but the molecular details of this inhibition remain elusive. Polymerase basic protein 2 (PB2) and nucleoprotein (NP) were suggested to be the possible target of Mx1, but a direct interaction between Mx1 and any of the viral proteins had not been reported. We demonstrated, for the first time, that Mx1 interacts with influenza NP and PB2 and that these interactions are associated with a strongly decreased interaction of NP with the viral polymerase complex. Inhibition of the NP–PB2 interaction is an active process requiring enzymatically active Mx1. Furthermore, we confirmed the importance of NP as determinant for Mx1 sensitivity. In a minireplicon system, avian-like NP was more sensitive to inhibition by murine Mx1 than NP from human influenza A virus isolates. Likewise, Mx1 displaced avian NP from the viral ribonucleoprotein complex more easily than human NP. The stronger resistance of the A/H1N1 pandemic 2009 virus against Mx1 also correlated with a reduced inhibition of the PB2–NP interaction. Our findings support a model in which Mx1 interacts with the influenza ribonucleoprotein complexes and interferes with its assembly by disturbing the PB2–NP interaction.

166 **Pandemic influenza A viruses escape from restriction by human MxA through adaptive mutations in the nucleoprotein**

Benjamin Mänz (1,2), Dominik Dornfeld (1), Veronika Götz (1), Roland Zell (3), Petra Zimmermann (1), Otto Haller (1), Georg Kochs (1) and Martin Schwemmle (1)
(1) University of Freiburg, Germany
(2) Erasmus Medical Center, Rotterdam, The Netherlands
(3) University of Jena, Germany

Influenza A viruses of avian or swine origin sporadically enter into the human population but do not transmit between individuals. In rare cases, however, they establish a new virus lineage in humans thereby causing devastating pandemics. The mechanisms by which invading viruses overcome the species barrier are not well understood, but multiple adaptations to the new host are required. In this study, we identified adaptive mutations in the nucleoprotein (NP) of pandemic strains A/Brevig Mission/1/1918 (1918) and A/Hamburg/4/2009 (pH1N1) that confer resistance to the interferon-induced dynamin-like MxA GTPase. These resistance-associated amino acids in NP differ between the two strains but form a similar discrete surface-exposed cluster in the body domain of NP, indicating that MxA resistance evolved independently. The 1918 cluster was conserved in all descendent strains of seasonal influenza viruses and is virtually absent in strains of avian origin. Introduction of this cluster into the NP of the MxA-sensitive influenza virus A/Thailand/1(KAN-1)/04 (H5N1) resulted in a gain of MxA resistance coupled with a decrease in viral replication fitness. Conversely, introduction of MxA-sensitive amino acids into pH1N1 NP enhanced viral growth in MxA-negative cells. Phylogenetic analysis revealed a positive selection of MxA resistance-enhancing NP mutations in the human host. We conclude that human MxA represents a barrier against zoonotic introduction of avian influenza viruses and that adaptive mutations in the viral NP should be carefully monitored.

167 **Chicken MDA5 senses short double-stranded RNA and is involved in interferon induction against influenza virus infection**

Takehiko Saito, Tsuyoshi Hayashi, Chiaki Watanabe, Yasushi Suzuki, Taichiro Tanikawa, Yuko Uchida *Influenza and Prion Disease Research Center National Institute of Animal Health*

Melanoma differentiation-associated gene-5 (MDA5) and retinoic acid inducible gene-I (RIG-I) sense double-stranded RNA (dsRNA) from various RNA viruses to induce anti-viral response in infected host cells. It has been indicated that chickens lack RIG-I that senses short dsRNA and is involved in interferon (IFN) response against influenza virus infection. Lack of RIG-I is suggested to attribute to sensitivity of chickens against highly pathogenic avian influenza viruses (HPAIVs). In this study, we examined whether chicken MDA5 (chMDA5) senses short dsRNA and induces IFN response upon AIV infection. chMDA5 gene was cloned and was transiently expressed in chicken cell lines. Chicken IFNβ (chIFNβ) mRNA was measured by real-time PCR after stimulation by short and long poly(I:C) as well as by infection with influenza viruses. siRNA designed against chMDA5 (siMDA5) was used to knock-down chMDA5 expression. Induction of chIFNβ mRNA in DF-1 cells by short poly(I:C) was almost completely abolished by siMDA5 whereas residual activity remained after stimulation by long poly(I:C). Short poly(I:C) induced chIFNβ mRNA in DF-1 cells transiently expressing chMDA5 to a greater extent than long poly(I:C). Transient expressed chMDA5 enhanced chIFNβ expression when infected with an AIV in DF-1 cells and with both an AIV and an HPAIV in LMH cells although suppression of virus replication was not observed in both cell lines. We demonstrated that chMDA5 preferentially senses short dsRNA and is involved in IFN response against AI viruses in chicken cell lines. This illustrates that chMDA5, in part, compensates functions of RIG-I in chickens.

168 **Humoral response at immunization with live and inactivated EBOV**

Alexander Chepurnov
Institute of clinical immunology Russian Academy of medical science Siberian branch

The heterologous immunoglobulins are known to be among the possible means of specific prophylaxis and treatment of viral infections. The choise of antigen for immunization in order to obtain the full-blooded immunoglobulins is very actual. It is obvious that the use of inactivated antigen simplifies the immunoglobulin obtaining but their quality must be evalueted. The investigation of properties of the sera received by immunization of equal quantities of live and inactivated Ebola virus (EV) which was preliminary concentrated by ultrafiltration and purified in sacharose gradient was performed. The sera obtained by immunization with live EV possessed the high neutralizing properties but showed the low titers in IEA. The sera obtained by immunization with inactivated EV possessed the low neutralizing properties but demonstrated the high titers in IEA. The western-blot analysis showed that the sera received by live EV immunization interacted mainly with surface glycoprotein and then in succession of decrease of molecular weight of EV proteins. The amounts of antibodies against separate EV proteins in sera received by inactivated EboV immunization were correlated with their proportions in virion.

169 **Degradation of RIG-I and inhibition of IFN-beta induction by Toscana Virus NSs protein.**

Maria Grazia Cusi, Gianni Gori Savellini, Chiara Terrosi, Barbara Martorelli
Department of Medical Biotechnologies, University of Siena, Italy

Toscana Virus (TOSV) is a Phlebovirus, belonging to the *Bunyaviridae* family, responsible for central nervous system (CNS) injury in humans. Recent studies have shown that many members of the *Bunyaviridae* family possess the NSs protein with a role in the escape to the antiviral system. TOSV, conversely to the other bunyaviruses, showed an IFN-b inducing activity, although having a non-structural protein NSs which is an IFN-? antagonist. Recent findings highlighted a significant decrease of RIG-I protein in cells over-expressing TOSV NSs, leading to a significant decrease of RIG-I-mediated IFN-b promoter activation. TOSV NSs was able to bind the N-terminal CARD domains of RIG-I, and appeared to mediate RIG-I degradation. It is not known whether it was due to a direct effect of NSs on RIG-I or to a proteasomal degradation. We show that the proteasome inhibitor MG-132 was able to partially restore RIG-I cellular levels and IFN-b promoter activation in cells expressing NSs, demonstrating the existence of an evasion mechanism based on inhibition of the RIG-I sensor. In order to detect the NSs domain(s) involved in this activity, the protein was modified by deletion of a C-terminal sequence (?NSs); this did not seem to affect the RIG-I level or interfere with RIG-I mediated IFN-? promoter activation. Further studies are in progress to understand NSs activity and its role in the innate immunity which may contribute to identify a novel mechanism for bunyaviruses by which TOSV NSs counteracts the early IFN response.

170 **Cytokine profil in Crimean-Congo Hemorrhagic fever**

Anna Papa (1), Katerina Tsergouli (1), Dilek Yağcı Çağlayık (2), Gulay Korukluoglu (2), Yavuz Uyar(2), Najada Como(3), Majlinda Kota(3), Eugena Tomini(3), Silvia Bino(3)
1 Dept of Microbiology, medical School, Aristotle University of Thessaloniki, Greece
2 Public Health Institute of Turkey, Microbiology Reference Laboratories Department, Turkey
3 Department of Epidemiology and Control for Infectious Diseases. Institute of Public Health, Albania.

Background: Crimean-Congo hemorrhagic fever (CCHF) is a viral disease with fatality 5-30%. Humans are infected through tick bite or by direct contact with blood or tissues of viremic patients or animals. The current knowledge about the immune response and pathogenesis of the disease is limited. In the present study we estimated the serum levels of various cytokines, chemokines and growth factors in acute CCHF human cases and correlate them with the disease severity and outcome.
Materials and Methods: Serum levels of 27 cytokines (IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, basic FGF, eotaxin, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1α, MIP-1β, PDGF-BB, RANTES, TNF-α, and VEGF) were measured simultaneously in 33 CCHF patients (11 male) aged 4-67 years (median 43) from Turkey, Albania and Greece. Two cases were fatal. The Bioplex suspension array system was used, and the statistical analysis was performed by SPSS.
Results: A strong immune response was observed, since the level of 21 cytokines were affected. IP-10 and RANTES were elevated in almost all cases, with significantly higher concentrations in the fatal and severe cases, together with MCP-1, IL-6, IL-8 and IL-10. Cytokines levels differ according to the day of illness, with increased VEGF, GM-CSF and MIP-1b in the third week of illness in the severe cases.
Conclusions: Many cytokines play a catalytic role in the course and outcome of CCHF. Understanding the host immune response to CCHFV will enable future therapeutic approaches for CCHF and viral hemorrhagic fevers in general.

171

A Comprehensive Proteomic View of the Suppression of Host Cell Antiviral Responses by Respiratory Syncytial Virus

Keyur Dave1, Alexander Bukreyev2, Emma Norris1, Ursula Buchholz2, Marcus Hastie1, Madeleine Headlam1, Buddhika Jayakody1, Kirsten Spann3, Cassandra Wright1, Toshna Singh1, Peter Collins2 and Jeffrey Gorman1
1Protein Discovery Centre, Queensland Institute of Medical Research, Brisbane, Australia, and 2Respiratory Virus Section, Laboratory of Infectious Diseases, National Institute for Allergy and Infectious Diseases, NIH, Bethesda, USA and 3Sir Albert Sakzewski Virus Research Centre, Brisbane, Australia.

Respiratory syncytial virus (RSV) is the most serious viral cause of lower respiratory tract infections in infants, young children and immunocompromised individuals. It is increasingly being recognised as a serious respiratory pathogen in the elderly. A hallmark of RSV is the lack of solid protection against re-infection, resulting in multiple infections throughout life. There are no vaccines or efficacious therapeutics for RSV. A variety of studies have been conducted in order to identify novel approaches for vaccine and antiviral design by gaining a better understanding of the interaction of RSV with host cells. We have conducted comprehensive proteomic analyses of RSV infected A549 cells to gain a better picture of the host cell response. Our studies have quantified 4,900 protein groups in wild-type RSV- and mock-infected A549 cells. Sixty-two of these protein groups were differentially regulated by infection using a false discovery rate cut-off of 1% for statistically significant regulation. Many of the regulated proteins are involved in interferon responses. One surprising finding was that RSV induces a variant of the interferon-induced MxA with a modified C-terminus which may be analogous to the production of a proviral form of this protein induced by herpes simplex virus-1. RSV encodes two small proteins which suppress antiviral responses of the host cell, including interferon induction and signalling. These proteins are termed non-structural proteins (NS1 and NS2) because they appear not to be packaged with virions. One of these proteins, NS1, appears to be the major culprit in terms of host cell response suppression, which it achieves through post-translational mechanisms. For instance it is proposed that NS1 acts as an E3 ligase. NS1 would appear to be a potential target for development of therapeutics for RSV. Accordingly, we have adopted a proteomic approach in conjunction with reverse viral genetics for identification of molecular targets for NS1 and to define its mechanism of action. Of interest is that NS1 appears to diminish the production of a proapoptotic proteome within the infected cell by promoting the survival of antiapoptotic proteins in order to suppress apoptosis on one hand whilst suppressing interferon responses on the other hand. Our studies also indicate that NS1 appears to suppress type II, in addition to types I and III, interferon signalling, suggesting that STAT1 may be a target of NS1. Processes used to achieve this novel marriage of reverse viral genetics and high-performance proteomic technologies will be presented in addition to the data supporting the observations cited above.

172 **Influenza virus infection induced Ebp1 modification**

Ayae Honda, Shinichiro Kume, Yukari Miyake, Koji Kadoi

Department of Frontier Bioscience, Hosei University

Influenza virus infection influences the gene expression of the cell, and after virus infection a big amount of viral genes express in the cell. We supposed that these changing in the cell may induce physical and chemical changing of cell, and these changing may induce the activation of cellular metabolism and consumption of energy. Whereas a big amount of energy will produce. Under this idea, the tool for measuring the temperature was developed polystylene bead with Rhodamine and measured the temperature of influenza virus infected and uninfected cells. Interestingly the temperature of influenza virus infected cell was risen about 4 K compared, however we could not detected any temperature rising on influenza virus uninfected cell. We also assayed the Pyruvate kinase expression in influenza virus infected cells. At 4 hpi the expression of pyruvate kinase was induced and consumption of ATP observed at 0hpi. We would like to discuss this result.

173 **Intrinsic and innate immune properties of PMLIV during VSV infection**

Faten El Asmi1,2, Mohamed Ali Maroui1,2, Nathalie Roders1,2, Danielle Blondel3, Sébastien Nisole2,4, and Mounira K. Chelbi-Alix1,2

1CNRS FRE3235, Paris, France, 2Université Paris Descartes, Paris, France, 3CNRS UPR 3296, Gif sur Yvette, France,4INSERM UMR-S 747, Paris, France.

Promyelocytic Leukemia (PML) protein, the organizer of small nuclear-matrix structures named nuclear bodies, has been implicated in the antiviral response toward diverse cytoplasmic replicating RNA viruses through different mechanisms. PML is covalently conjugated to the small ubiquitin-like modifier (SUMO). Splice variants transcribed from the *PML* gene yield several PML isoforms (PMLI to PMLVII) that share the same N-terminal region but differ in their C-termini, thus conferring the specific functions of each. *PML* knock out mice are more sensitive to vesicular stomatitis virus (VSV). These *in vivo* observations corroborate with our previous findings that PMLIII confers resistance to VSV. The comparative study performed with cells stably expressing each of PML isoform revealed that only PMLIII and PMLIV confer resistance to VSV, whereas other nuclear (PMLI, II, V and VI) and cytoplasmic (PMLVII) PML isoforms fail to do so. The antiviral activity of PMLIV is higher than that of PMLIII and is not saturable by an excess of virus. Unlike PMLIII which anti-VSV activity is strictly IFN-independent, PMLIV can act at two stages of VSV infection: it inhibits viral mRNA and protein synthesis early after cell infection and also triggers innate immunity, leading, *via* IRF3 activation, to IFN-b synthesis, which can protect other cells from viral infection. The SUMOylation of PMLIV is required for both its direct antiviral activity and the activation of IFN response. Our results show for the first time that PMLIV is implicated in both anti-VSV intrinsic and innate immunity

174

Antigen editing by monocyte-derived dendritic cells in the lung shapes the quality of anti-influenza T cell immunity

Jazmina L.G. Cruz, Anja Lüdtke, Sergio Gómez-Medina, José V. Pérez-Girón and César Muñoz-Fontela
Heinrich Pette Institute, Leibniz Institute for Experimental Virology, Hamburg, Germany.

Circulating monocytes infiltrate peripheral tissues after injury or infection where they show enormous plasticity to differentiate into macrophages and monocyte-derived dendritic cells (moDCs). However, the contribution of moDCs to the initiation of immunity to viruses is poorly understood with only a few reports indicating their influence on immunopathology. In this study, we have investigated the role of moDCs on the immune response to pulmonary influenza virus infection. After infection, blood-borne monocytes infiltrated the lung where they became fully differentiated DCs. Interestingly, moDCs did not follow the classic DCs paradigm of peripheral DCs, which acquire antigens and then migrate into lymphoid tissues to initiate adaptive immunity. Conversely, moDCs captured most of the viral antigen in the lung, but only antigen-loaded CD103+DCs, migrated to the lung-draining mediastinal lymph nodes. To investigate putative antigenic transfer between moDCs and CD103+ DCs we utilized bone marrow chimeras to follow the fate of MHC-peptide complexes in the lung during infection. We demonstrated exchange of preformed MHC-I-peptide complexes between lung DCs on influenza-infected animals, which strongly suggested that MHC-peptide transfer served to edit the antigenic peptide repertoire to be transported by migratory DCs to the lymphoid tissues. Further pointing out to this direction, specific depletion of moDCs in vivo reduced the clonal expansion of influenza-specific CD8 T cells. Taken together, our findings suggest a role of moDCs as lung antigenic depots, providing migratory DCs with a source of processed MHC-peptide complexes thereby enriching the viral peptide repertoire presented to naive T cells at the sites of priming.

175

Both the C and V proteins of canine distemper virus play essential roles for the virus replication in human epithelial cells

Noriyuki Otsuki(1, Tsuyoshi Sekizuka(2, Toru Kubota(1, Yuichiro Nakatsu(1, Fumio Seki(1, Kouji Sakai(1, Ryoji Yamaguchi(3, Hideo Fukuhara(4, Katsumi Maenaka(4, Makoto Kuroda(2, Makoto Takeda(1
(1: Department of Virology 3, National Institute of Infectious Diseases, Tokyo, Japan
(2: Laboratory of Bacterial Genomics, Pathogen Genomics Center, National Institute of Infectious Diseases, Tokyo, Japan
(3: Department of Veterinary Pathology, Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan
(4: Laboratory of Biomolecular Science, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan

Canine distemper virus (CDV) has recently expanded its host ranges to non-human primates. The replication potential of CDV in humans is unclear. Our study revealed that CDV possesses an ability to use human nectine4 as a receptor. However, the Ac96I and 007Lm CDV strains, which were isolated using canine SLAM-expressing Vero cells, failed to replicate in nectin4+ human epithelial H358 cells. After eight passages in H358 cells, they have adapted to replicate well in H358 cells. Deep sequencing analyses suggested that the original Ac96I and 007Lm strains (Ac96I-VDS and 007Lm-VDS, respectively) had defects in the C and V proteins, respectively, and subpopulations with the repaired C and V proteins (Ac96I-p8 and 007Lm-p8) were selected during the 8 passages in H358 cells. The functions of the CDV V proteins of 007Lm-VDS and 007Lm-p8 (VDS-V and p8-V, respectively) were analyzed using H358 cells that were engineered to constitutively express the VDS-V and p8-V proteins (H358/VDS-V and H358/p8-V cells, respectively). Upon infection with 007Lm-VDS, nuclear translocation of IRF3 was observed in both H358/VDS-V and H358/p8-V cells. On the other hand, the IFN-mediated transcription of ISG15, OAS1 and ISG56 was completely blocked in H358/p8-V cells, but fully stimulated in H358/VDS-V cells. These data revealed that both the C and V proteins of CDV are functional to counteract the human IFN system and critical for CDV replication in human epithelial cells.

176 **Responses to Pneumococcus in HumangInfluenza infection Directly Alters Innate IL-23 and IL-12p70 and Subsequent IL-17A and IFN-**

1Sinead Loughran*, 1Patrick Power*,1Samantha McQuaid, 2Ingileif Jonsdottir, 1Richard Lalor and 1Patricia A Johnson
1Viral Immunology laboratory, School of Nursing and Human Sciences, Dublin City University, Glasnevin, Dublin 9. Ireland. 2Department of Immunology, Landspitali, The National University Hospital of Iceland, Reykjavik, Iceland

It is well accepted that influenza A virus predisposes individuals to often more severe superinfections with streptococcus pneumonia. However the mechanisms that lead to this synergy are not clearly understood. Recent data suggests that competent TH17 immunity is crucial to clearance and protection from invasive pneumococcal disease of the lung. This study therefore aims to identify the modulation by influenza of key human innate and adaptive immune responses to pneumococcus. Human monocytes were obtained from buffy coats of healthy donors and exposed to influenza (IAV), or Influenza haemagglutinin (HA), or pneumococcus alone or in combination. Allogeneic-T cells from different donors were added to the infected or treated monocytes and incubated at 37°C for 3-days. Human cytokine levels in supernatants were quantified by ELISA. in allogeneic mixed lymphocyte cultures.gWe demonstrate that early IAV infection significantly reduced levels of pneumococcus driven 12p70, IL-23 and IL-27 in human monocytes with significant impairment of pneumococcus induction of IL-17A and IFN- We also provide evidence to suggest that the HA component of the virus is at least partially responsible for this downward pressure on IL-17 responses but surprisingly this suppression by HA occurs despite robust IL-23 levels in HA treated monocyte cultures. This study improves our understanding of influenza-pneumococcal co pathogenesis in adults and may explain the enhanced colonization with S pneumoniae in children associated with early influenza infection

177 **Prevalence and predictors for heterosubtypic antibodies against influenza A in Humans**

Ines Kohler, Alexandra U. Scherer, Osvaldo Zagordi, Arkadiusz Wyrzucki, Matteo Bianchi, Marco Steck, Bruno Ledergerber, Huldrych F. Günthard and Lars Hangartner
1. Institute of Medical Virology, University of Zurich, Zurich
2. Klinik für Infektionskrankheiten und Spitalhygiene, University Hospital, Zürich

Originally, the subtypes of influenza A have been defined as serotypes, indicating that there are no or only very few shared antibody epitopes. Indeed, the most immunogenic areas of influenza hemagglutinin were later found to co-localize with the most variable areas of the protein. It is therefore not surprising that antibody responses to hemagglutinin only recognize the eliciting or closely related strains. However, since the early 90ies, several heterosubtypic antibodies, i.e. antibodies that neutralize more than one subtype of influenza, have been isolated. Most of these bind to a conserved epitope in the stem of the influenza hemagglutinin. In an epidemiological study, we have analyzed the presence of binding and neutralizing heterosubtypic antibodies in the serum from 273 random donors. We have found that most individual possess heterosubtypic antibodies, and that those increase with age. Moreover, we could show that concomitantly high binding titers to H1 and H3 were predictive for high titers against heterotypic isolates. Univariate analysis also demonstrated that repetitive vaccination favors the development of heterosubtypic antibody titers. These findings indicate that any novel vaccination strategy targeting the conserved epitopes of influenza hemagglutinin could profit from pre-existing antigen-experienced B cells.

178 Inhibition of Borna disease virus replication by an endogenous bornavirus element in ground squirrel genome

Kan Fujino, Masayuki Horie, Tomoyuki Honda, Keizo Tomonaga
Department of Viral Oncology, Institute for Virus Research, Kyoto University

Animal genomes contain endogenous viral sequences, such as endogenous retroviruses. Recently, we and others discovered that non-retroviral viruses (NRV) have also been endogenized in many vertebrate genomes. Bornavirus belongs to the Mononegavirales and have left endogenous elements, called EBLN (endogenous bornavirus-like nucleoprotein), in the genomes of many mammals, including us. The striking features of EBLN are that they hold relatively long open reading frame and show high sequence homology to the nucleoprotein (N) of current bornaviruses. Furthermore, it has been known that some EBLNs are transcribed as mRNA. These features of EBLNs provide us to speculate that the EBLNs might have functions in the cells as adopted genes. The EBLN in the thirteen-lined ground squirrel (*Ictidomys tridecemlineatus*) genome, itEBLN, is the one of the most intriguing EBLNs, because itEBLN exhibits 77% sequence identity to bornavirus N and is considered to be generated by a very recent integration event. In this study, to analyze the possible function of itEBLN, we revived itEBLN from the ground squirrel genomes and investigated the roles of the revived protein in Borna disease virus (BDV) replication. Interestingly, itEBLN, but not human EBLNs, co-localized with the viral factory of BDV in nucleus. In addition, itEBLN appeared to affect BDV polymerase activity by being incorporated into the viral ribonucleoprotein. Moreover, cell lines stably expressing itEBLN showed the resistance to BDV infection. Our study suggested that, as with some endogenous retroviruses, the endogenous RNA viral elements may also have potential to inhibit the infection of related exogenous viruses.

179 Systematic Identification and Characterization of Interferon-Induced Antiviral Factors

Yao Wang, Su-Yang Liu, Roghiyh Aliyari, Genhong Cheng
Department of Microbiology, Immunology and Molecular Genetics,
University of California, Los Angeles, USA

Innate immunity is the host's first line of defense against pathogen invasion. Type I interferons including IFNα, IFNβ and IFNω are key players in the innate immune responses against viral infection. In the recent years, pathways leading to the induction of type I IFNs have been extensively studied, and it is known that hundreds of genes are activated as a result of IFN signaling. These genes, collectively called Interferon-Stimulated Genes (ISGs), act in concert to establish an antiviral state in the cell. However, except for a handful of examples such as Mx1, ISG20, PKR, Tetherin, and the IFITM family, the functions of the vast majority of ISGs remain largely uncharacterized. We have performed a microarray analysis to identify ISGs that are specifically up-regulated by type I IFNs in murine bone marrow-derived macrophages. Adopting an overexpression screening approach, we have systematically evaluated the potential roles of these individual ISGs in inhibiting different viruses including vesicular stomatitis virus (VSV), murine herpesvirus 68 (MHV-68), and influenza virus. To prioritize the most biologically relevant candidates, we have integrated data from our protein-protein interaction screens using viral proteins as bait as well as previous genome-wide siRNA screens and genome-wide genetic association studies in the literature. Subsequently, the exact antiviral mechanisms of the prioritized candidates are further dissected using assays that focus on specific steps of the viral life cycle such as viral entry, transcription, replication, and budding. Here, we will present our characterization of selected candidates.

180 **Interferon (IFN) induces an antiviral state in cells that results in alterations of the patterns**

Jelena Andrejeva (1), Hanna Norsted (1), Stephen Goodbourn (2), Rick Randall (1)
(1) University of St. Andrews, School of Biology, BSRC, St. Andrews, UK
(2) Biochemistry and Immunology, Department of Basic Medical Sciences, St. George's Hospital Medical School, University of London, London, UK

Interferon (IFN) induces an antiviral state in cells that results in alterations of the patterns and levels of parainfluenza virus type 5 (PIV5) transcripts and proteins. This study reports that IFN-stimulated gene 56/IFN-induced protein with tetratricopeptide repeats 1 (ISG56/IFIT1) is primarily responsible for these effects of IFN. It was shown that treating cells with IFN after infection resulted in an increase in virus transcription but an overall decrease in virus protein synthesis. As there was no obvious decrease in the overall levels of cellular protein synthesis in infected cells treated with IFN, these results suggested that ISG56/IFIT1 selectively inhibits the translation of viral mRNAs. This conclusion was supported by in vitro translation studies. Previous work has shown that ISG56/IFIT1 can restrict the replication of viruses lacking a 2'-O-methyltransferase activity, an enzyme that methylates the 2'-hydroxyl group of ribose sugars in the 5'-cap structures of mRNA. However, the data in the current study strongly suggested that PIV5 mRNAs are methylated at the 2'-hydroxyl group and thus that ISG56/IFIT1 selectively inhibits the translation of PIV5 mRNA by some as yet unrecognized mechanism. It was also shown that ISG56/IFIT1 is primarily responsible for the IFN-induced inhibition of PIV5.

181 **Interactions between Natural Killer cells and antigen presenting cells during the infection by Lassa virus**

Marion Russier, Stéphanie Reynard, Sylvain Baize
1) Unité de Biologie des Infections Virales Emergentes, Institut Pasteur - Lyon, France
2) International Center for Infectiology, Université de Lyon - Lyon, France

Lassa virus (LASV) is an Arenavirus that can be responsible for a hemorrhagic fever in West Africa. The immune responses and the pathogenesis associated with this disease are unknown. LASV displays tropism for antigen presenting cells (APC) such as dendritic cells (DC) and macrophages. Natural Killer (NK) cells are involved in the clearance of infected cells and promote optimal immune responses by interacting with APC. We used an in vitro model of human NK and autologous APC coculture to study the role of NK cells and to characterize their interactions with APC during LASV infection. In addition, NK cell responses developed after LASV infection were compared with those induced by Mopeia virus infection (MOPV), closely related to LASV but not pathogenic for humans. Cell activation, cytokine production, proliferation and NK cell-mediated killing were analyzed. We have shown that NK cells are activated, proliferate and have increased cytotoxic capacity in the presence of LASV- or MOPV-infected macrophages, but not DC. This process involves cell contacts and type I IFN. However, these cells are neither able to kill the infected cells nor produce IFN-γ. The importance of type I IFN response has also been further analyzed using recombinant LASV holding mutations into the nucleoprotein that abolish its ability to inhibit type I IFN production. These results bring substantial advances in the understanding of innate immunity during Lassa fever.

182 **Variation in the interferon response induced by influenza A virus strains.**

Konrad Bradley, Lorian Hartgroves, Ruth Elderfield, Holly Shelton and Wendy Barclay. *Imperial College London*

Like many human pathogens, seasonal influenza A viruses modulate the innate immune response to facilitate viral replication in the host. However, not all strains of influenza virus equally control innate immune responses, as avian H5N1 viruses isolated from human patients can cause an increased cytokine response, frequently referred to as a "cytokine storm." Previously, we showed that an early viral isolate from the 2009 H1N1 pandemic induced a greater cytokine response than a representative seasonal H3N2 isolate and this was partly accounted for by differences in the ability of the NS1 proteins to control interferon promoter activation. In this research, we aimed to understand differences in the epithelial cytokine response driven by influenza A virus strains including H5N1. We created a set of viruses that shared the same HA and NA genes but had polymerase complex and NS genes from seasonal H3N2, pandemic H1N1 or avian H5N1 influenza strains, so that any differences in interferon induction would be determined by intracellular events and not by differences in receptor use or kinetics of virus entry. These viruses showed profound differences in the extent and timing of activation of the interferon promoter and also in the outcome of infection in mice with the H5N1 RG virus inducing the most rapid response. This research aims to reveal valuable insights into viral genetic variations responsible for the high induction of cytokines, and whether mutations in the immune modulating proteins might overcome such a defect to promote a more human adapted virus.

183 **Deep sequencing analysis of defective genomes of parainfluenza virus 5 and their role in interferon induction.**

Marian Killip (1), Dan Young (1), Derek Gatherer (2), Craig Ross (3), Andrew Davison (2), Steve Goodbourn (3), Richard Randall (1)
(1) Centre for Biomolecular Sciences, University of St Andrews, UK.
(2) Centre for Virus Research, University of Glasgow, UK.
(3) Division of Basic Medical Sciences, St George's, University of London, UK.

Parainfluenza virus 5 (PIV5) preparations generated by high multiplicity passage are potent activators of the interferon (IFN) induction cascade due to the accumulation of defective interfering (DI) viruses. Nucleocapsid RNA from these virus preparations was analysed by deep sequencing to examine the range of DIs present, and to approximately quantify the ratio of defective to non-defective genomes. Genome sequence coverage of IFN-inducing PIV5 wildtype (wt) and PIV5-VΔC (which lacks a functional V protein) was enriched in the trailer region, consistent with an accumulation of trailer copyback (TrCB) genomes. We used bioinformatics to identify the join points associated with the different TrCB species present and to determine their relative frequencies. We also identified potential leader copyback, internal deletion and internal duplication genomes, however these species did not contribute significantly to IFN induction. DI-rich PIV5 wt preparations strongly activate the IFN induction cascade despite encoding the V protein, an efficient inhibitor of the IFN response. We demonstrate that non-defective PIV5 wt cannot prevent activation of the IFN response by co-infecting TrCBs due to the interfering effects of TrCBs on non-defective virus protein expression. Consequently, TrCBs rapidly activate the IFN induction cascade prior to the expression of detectable levels of V by co-infecting non-defective virus.

184 **RVFV growth in human macrophages is severely restricted by knockout of a PSL motif in the virus NSs virulence factor**

Anita K McElroy, Kimberly A Dodd, Stuart T Nichol and Christina F Spiropoulou
1. US Centers for Disease Control and Prevention, Viral Special Pathogens Branch (all authors)
2. Emory University School of Medicine, Division of Pediatric Infectious Disease (McElroy)
3. University of California Davis, School of Veterinary Medicine (Dodd)

Rift Valley Fever virus (RVFV) is a mosquito borne pathogen of animals and humans; its major virulence factor is the NSs protein. NSs mediates host-cell transcription shut-off and interferon antagonism. NSs abrogates interferon and cytokine induction in human macrophages leading to rapid virus growth in these critical immune cells. An alignment of the RVFV NSs with other interferon antagonists revealed a conserved Proline-Serine-Leucine (PSL) motif. In this study, we sought to determine if this PSL motif contributed to pathogenesis in macrophages. Recombinant RVFV containing a mutation of the NSs PSL motif to Alanine-Alanine-Alanine (AAA) or deletion of the entire NSs ORF (Del NSs) were generated using reverse genetics. Surprisingly, the AAA virus was more attenuated in macrophages than a virus lacking the entire NSs protein. In contrast, AAA grew equivalent to wild-type (WT) in several commonly used laboratory cell lines. The attenuation of AAA in macrophages compared to Del NSs was not mediated by enhanced IFN induction, as significantly more IFN was produced in cells infected with Del NSs. Further investigation of the AAA phenotype revealed that the block to AAA virus growth was at the level of viral replication. To determine the mechanism responsible for this replication block, we defined the protein/protein interactions that occur with the AAA NSs protein. We constructed recombinant tagged AAA and WT NSs proteins and identified 29 proteins that specifically interact with the AAA NSs. These proteins are components of several different cellular processes including the innate anti-viral response and the ubiquitination/degradation pathway.

185 **Interferon-inducible antiviral protein Tetherin/BST-2 inhibits Hazara virus Replication**

Yohei Kurosaki1,2, Akiko Nishimura2, Shuzo Urata1 and Jiro Yasuda1,2.
1Department of Emerging Infectious Diseases, Institute of Tropical Medicine, Nagasaki University
2First Department of Forensic Science, National Research Institute of Police Science.

Hazara virus (HAZV) is a member of the genus *Nairovirus* of the family *Bunyaviridae*. HAZV is closely related to Crimean-Congo hemorrhagic fever (CCHF) virus and these viruses share the same serogroup. HAZV is nonpathogenic for human and can be handled in low containment level-2 facilities. Therefore, HAZV has been employed as a model to study CCHF virus. Tetherin, also known as BST-2, is an interferon (IFN)-inducible type II membrane protein that can act as a restriction factor against a number of enveloped viruses. Here we investigated the antiviral activities of Tetherin against HAZV. We first examined the effects of human Tetherin expression on HAZV replication. The stable expression of Tetherin in SW13 (human adrenocarcinoma) cells resulted in significant reduction of both viral RNA and titer in the culture supernatants at 48h post-infection. This result suggested that Tetherin had an inhibitory effect for the production of HAZV. It was also shown that IFNα inhibited the growth of HAZV in SW13 cells. The specific depletion of Tetherin using siRNA partially rescued the inhibition of HAZV production by IFNα, suggesting that Tetherin may play any role in inhibiting HAZV replication by IFNα as an antiviral interferon stimulating gene. HAZV did not affect the expression level of Tetherin in the infected cells, indicating that HAZV did not encode any antagonist against Tetherin. These results suggest that Tetherin may be useful for the establishment of a novel antiviral strategy against Nairoviruses.

186 **Interaction between human parainfluenza virus type 2 V protein and tetherin, an antiviral host factor**

Keisuke Ohta, Hideo Goto, Natsuko Yumine, Machiko Nishio
Department of Microbiology, Wakayama Medical University, Japan

Tetherin, also known as BST-2/CD317/HM1.24, is a cellular membrane protein that restricts budding of a number of enveloped viruses, including HIV-1/2, SIV and Ebola virus. These viruses have evolved specific countermeasures to overcome restriction by tetherin, via their envelope glycoproteins. The accessory V protein of human parainfluenza virus type 2 (hPIV-2, another enveloped virus, of the *Paramyxoviridae*) is a multifunctional protein that induces STAT protein degradation and the blockage of TLR7/9-dependent signalling, but little is known about the relationship between hPIV-2 and tetherin. Recently, using immunoprecipitation, we found that V interacts with tetherin, suggesting that V is a tetherin antagonist even though it is not an envelope protein. The hPIV-2 P protein, whose N-terminal domain is common with that of V, did not bind tetherin, indicating that the C-terminal V-specific region of V interacts with tetherin. Tetherin is a type II transmembrane protein containing of an N-terminal cytoplasmic tail, an extracellular domain, and a C-terminal glycosyl phosphatidylinositol membrane anchor (GPI). The deletion of its GPI disrupted its interaction with V, suggesting that the GPI is critical for V binding. V apparently binds directly to the GPI of tetherin, and hPIV-2 appears to antagonize tetherin by a different mechanism from other enveloped viruses.

187 **Inhibition of NF-κB activation by peptide derived from nucleoprotein of Borna disease virus**

Akiko Makino, Kan Fujino, Yuya Hirai, Kozue Sofuku, Shoko Nakamura, Shohei Kojima, Tomoyuki Honda, Keizo Tomonaga
Department of Viral Oncology, Institute for Virus Research, Kyoto University

Borna disease virus (BDV), which has broad host range in vertebrates, causes persistent infection in nucleus that can lead to neural disorder in horses and sheep. Constitutive activation of NF-κB suppresses the BDV growth, however, NF-κB pathway is not activated in the acute infection of BDV (Bourteele, 2005). Here, to elucidate mechanism for the inhibition of NF-κB activation by BDV infection, we evaluated cross-talk between BDV infection and NF-κB pathway.
In THP1-CD14 cells, which has SEAP gene as a NF-κB reporter, BDV infection did not increase SEAP activity from 12 hour to 2 weeks post infection, compared with TLR ligand stimulation. This result is consistent with previous report and suggests BDV has gene(s) that inhibits the activation of NF-κB. To discover common motifs between the amino acid sequences of BDV genes and NF-κB family, we performed Multiple EN for Motif Elicitation analysis and found that nucleoprotein of BDV (BDV-N) and NF-κB1 possess a common ankyrin-like motif. In THP1-CD14 cells, pre-treatment with the ankyrin-like peptide of BDV-N suppressed the SEAP activation by the stimulation with TLR ligands, while control peptide had no effect on it. NF-κB1 is phosphorylated by signal transduction and its C-terminal region containing ankyrin repeat undergoes selective degradation by proteasome pathway. Immunoprecipitation assay showed BDV-N was co-precipitated with NF-κB1, suggesting the possibility this interaction might inhibit NF-κB1 degradation. These results indicate BDV inhibits NF-κB activation via BDV-N, resulting in its benefit for BDV persistent infection.

188 **The never-ending battle between the Innate Immune System and the viruses: Influenza A NS1 protein**

Jessica Guerra, Stéphanie Anchisi, Dominique Garcin
Dpt. Microbiology and Molecular Medicine, School of Medicine. Geneva Switzerland.

Following infection with a virus, the body mounts an innate and adaptive immune response. IFNβ is one of the first molecules produced to fight against viruses once specific receptors, like RIG-I like receptors (RLRs) detect them. When the IFN system works correctly, is able to counteract almost 99% of viral infections. However, it rarely works rightly because, in order to successfully spread among their hosts, viruses developed different strategies to counteract at different levels the innate immune system (1). Influenza A NS1 protein has been described as the anti-innate immune viral factor responsible for the block in IFNβ production upon infection. Its interaction with the E3 ligase TRIM25 blocks RIG-I K63 poly-ubiquitination shutting down the IFNβ system. Despite this knowledge, the reason why NS1 needs to bind RNA to function is still unclear. Therefore we aim with this work to elucidate the contribution of the dsRNA binding ability of NS1 vs the protein-protein interaction to its mechanism of action. We confirmed the ability of the protein to down-regulate RIG-I signaling pathway and the importance of its RNA binding domain and effector domain for its full activity. The crucial role of both domains in the binding to 5'ppp dsRNA suggests that NS1 does more than simply sequestering the RNA from detection in the cytoplasm. Moreover preliminary data show an interaction between NS1 and RIG-I, which is still under investigation to clarify if it is TRIM25 mediated and for the first time what is the role of the RNA in this scenario. 1. Type 1 Interferons and the Virus-Host Relationship: A lesson in Détente. *Science 312, 879 (2006)*

189 **Mononegavirales leader RNA as agonist of RIG-I**

LOUBER Jade(1), BLOYET Louis-Marie(1), KOWALINSKI Eva(2,3), CUSACK Stephen(2,3), GERLIER Denis(1)
(1) Centre International de Recherche en Infectiologie, CIRI, INSERM U111, CNRS UMR5308, Université Lyon 1, ENS Lyon, 21 Avenue Tony Garnier, 69007 Lyon, France.
(2) European Laboratory of Molecular Biology, Grenoble area, 6 rue Jules Horowitz, BP 181, 38042 Grenoble Cedex 9, France.
(3) Virus Cell-host Interactions Unit, UJF-EMBL-CNRS, UMI 3265, 6 rue Jules Horowitz, BP 181, 38042 Grenoble Cedex 9, France.

Host defence against viruses depends on the rapid triggering of innate immunity through the induction of type I interferon (IFN) response. To this end, dedicated receptors are able to detect microbe-associated molecular patterns. RIG-I can sense viral RNA into the cytoplasm and then induce the production of IFN. Studies performed with in vitro synthesized RNA indicate that RIG-I responds to double strand RNA (dsRNA) with a free triphosphorylated 5'-end (5'ppp). However, the identity of viral RNA(s) recognized by RIG-I during an infection remains unclear. We show that synthetic leader RNA of a filoviridae (Ebola virus), a rhabdoviridae (Rabies virus) and a paramyxoviridae (Measles virus) can bind RIG-I in vitro. In Huh7.5 cells that express an inactive RIG-I mutant, the transfection of the three synthetic leader RNAs activates the IFN-beta promoter only when an active RIG-I is expressed. Binding of synthetic leader RNA of Ebola, Rabies and Measles viruses by RIG-I was detected in cellula. Indeed, synthetic leader RNAs were selectively immunoprecipitated with RIG-I. Furthermore, during activation of RIG-I by a synthetic 5'ppp-dsRNA or by polyinosinic:polycytidylic acid, a dsRNA analogue, no oligomerization of RIG-I could be observed by co-immunoprecipitation. These results suggest that leader RNA of Mononegavirales can be good RIG-I agonists without evidence of intracellular oligomerization of RIG-I.

190 **Different production of a viral RNA species between Sendai virus strains causes their remarkable difference in IFN inducibility**

Asuka Yoshida, Ryoko Kawabata, Takemasa Sakaguchi, Takashi Irie
Department of Virology, Institute of Biomedical & Health Sciences, Hiroshima University, Japan

Sendai virus (SeV), a prototype of paramyxoviruses, is one of the viruses that have been initially-revealedits antagonistic action against host innate immunity in which virus-derived RNA species are recognized by RIG-I-like receptors (RLRs), such as RIG-I and MDA5, followed by induction of interferon (IFN) -beta and a number of antiviral proteins. SeV accessory proteins C and V produced as P gene products have been shown to play key roles in the antagonism in multiple ways. In contrast, SeV has been used as "an IFN-inducing agent" in a number of studies. No reasonable explanation for this discrepancy has been provided yet. Only an obvious difference between the viruses with opposing characters is strains; one of the SeV strains mostly used as an IFN-antagonizing virus is Z, and that used as an IFN-inducing virus is Cantell. Here, we compared their abilities to antagonize the innate immune system and to induce IFN-beta production followed by establishment of antiviral state. Indeed, a remarkable difference in IFN-beta induction was there between the cDNA-derived Z and commercially supplied Cantell strains propagated once in eggs. Further studies using cloned viruses by a three-step limiting dilution revealed that a virus-derived RNA species but not loss of the antagonistic function of C and V proteins against the innate immune system primarily causes the marked IFN-beta inducibility of Cantell. Such difference in production of the RNA species between the strains might imply existence of a viral function to suppress certain viral RNA species for hiding from recognition by RLRs.

191 **Measles Virus Neutralization: Genotype-dependent neutralization epitopes in the Hemagglutinin protein which are immunogenic in natural infection and vaccination and elicit a long-term B cell memory.**

Miguel Ángel Muñoz-Alía (1,2), César Santiago (2), José M. Casasnovas (2), María L. Celma (1) y Rafaél Fernández-Muñoz (1).
1. Unidad de Virología, Hospital Ramón y Cajal, Madrid, Spain
2. Departamento de Estructura de Macromoléculas, Centro Nacional de Biotecnología(CNB-CSIC),Campus Cantoblanco, Madrid , Spain

At present Measles virus (MV), despite the availability of an attenuated vaccine, still causes over one hundred thousand deaths yearly. Although MV is serologically monotypic, 24 genotypes have been recognized so far which have shown different geographical and temporal distribution. We have previously observed genotype-dependent MV neutralization epitopes defined by murine anti-MV-H MoAbs. To know whether these epitopes were immunogenic in natural infection or vaccination, we have developed a competition binding radio-immunoassay on MV-H protein in its native context. For this purpose we established MV steady-state persistent infection by MV primary isolates belonging to the main circulating genotypes which express on its surface native oligomeric complexes of MV-H and MV-F glycoproteins. On these cells we measured binding of a number of (I125)-labeled anti-MV-H MoAbs and concentration-dependent competition by human sera from individuals with past infection by MV strains, isolated, sequenced and genotyped years before in our laboratory. We have identified some epitopes in MV-H that selectively bind to immunoglobulines from individuals with past infection by MV belonging to the same genotype. These epitopes were mapped after obtaining neutralization escape mutants and structural characterization. Thus, these genotype-dependent neutralization epitopes are immunogenic in natural infection and may elicit a long-term B cell memory. This procedure may allow, by using serum-libraries and a set of labeled anti-MV-H MoAbs, to find out retrospectively whether one MV genotype circulated in a geographic area at a given time. This information would be relevant to design improved measles vaccines, and therapeutic broad-neutralizing anti-MV antibodies.

192 **PPRV, The role of its accessory proteins in the inhibition of the induction of IFNβ**

Beatriz Sanz Bernardo1, Stephen Goodbourn2, Michael D Baron1
1 The Pirbright Institute, Surrey, UK
2 St George's Hospital Medical School, University of London, UK

Peste des petits ruminants virus (PPRV) is a morbillivirus that causes clinical disease in sheep and goats. It produces pneumoenteritis, gastritis and immunosuppression, and has a major, and growing, economic impact on families in underdeveloped countries. We are studying how PPRV interferes with the induction of IFNβ, an immediate cell response to virus infection. Infection with PPRV itself does not induce the activation of the IFN β promoter in Vero cells during the first 24 hours. This appears to be only in part due to active suppression of IFN induction; while the induction of IFNβ was lower in PPRV-infected cells, whether this induction was through the MDA-5 pathway (transfection with poly(I:C)) or the RIG-I pathway (infection with DI-containing Sendai virus), the effect of prior PPRV infection was minor. The effect of PPRV was greater in goat fibroblasts, suggesting a species specificity. The inhibition of poly(I:C)-induced IFNβ induction can be reproduced when the viral accessory V protein is expressed in transfected cells, and immunoprecipitation assays showed that V directly binds to MDA-5, as shown for the V proteins of other paramyxoviruses. The mechanism by which PPRV affects the RIG-I pathway is unclear: neither of the viral accessory proteins appear to bind RIG-I but each protein, like the virus, has a small effect on the induction of IFNβ through RIG-I. These data suggest that PPRV evades the cell response to infection as it doesn't induce IFNβ in infected cells and this may be partly linked to activity of its V protein.

193 **Respiratory syncytial virus induces but antagonises innate antiviral responses in well-differentiated paediatric primary bronchi**

Rémi Villenave1, Lindsay Broadbent1, Isobel Douglas2, Jeremy D. Lyons2, Peter V. Coyle3, Michael N. Teng4, Ralph A. Tripp5, Liam G. Heaney1, Michael D. Shields1,2, Ultan F. Power1
1 Centre for Infection & Immunity, School of Medicine, Dentistry & Biomedical Sciences, Queens University Belfast; 2 The Royal Belfast Hospital for Sick Children, Northern Ireland; 3 The Regional Virus Laboratory, Belfast Trust, Belfast, Northern Ireland; 4 Morsani College of Medicine, University of South Florida, Tampa, FL 33647, USA; 5 Department of Infectious Diseases, University of Georgia, Athens, GA 30602, USA.

RSV is the principal cause of severe lung disease in young infants. However, RSV pathogenesis is poorly understood. We recently reported an RSV infection model based on well-differentiated primary paediatric bronchial epithelial cells (WD-PBECs), which reproduced several hallmarks of RSV infection (Villenave et al, PNAS 109:5040-5, 2012). We therefore exploited this model to study components of innate immune responses to RSV. RSV infection virtually abrogated super-infection with Sendai virus and reduced super-infection with a different RSV strain. This was mediated, in part, by secreted factors. However, our data indicated type I IFNs were not implicated. Alternatively, IL-29 was, as it was detected in BALs and basolateral medium from RSV-infected infants and WD-PBECs, respectively, and IL-29 pre-treatment reduced SeV growth in WD-PBECs in a dose-dependent manner. In contrast, neither secreted factors nor IL-29, at doses found in basolateral medium from RSV-infected WD-PBECs, prevented RSV replication in pre-treated WD-PBECs. Using an RSV ΔNS1/NS2 deletion mutant, we demonstrated that RSV NS1/2 proteins are essential for RSV replication in WD-PBECs and implicated in antagonising IL-29-induced antiviral responses in Vero cells. Intriguingly, RSV-infected cells in WD-PBECs demonstrated massive reductions in Mx1 expression. Therefore, RSV induces but effectively antagonises antiviral innate immune responses in WD-PBECs.

194 **An UNBIASSED GENETIC SCREEN REVEALS the POLYGENIC NATURE of the INFLUENZA VIRUS ANTI-INTERFERON RESPONSE**

Maite Pérez-Cidoncha1 (1,4), Marian J. Killip (2), Juan C. Oliveros (1), Víctor Asensio (3,4), José A. Bengoechea (3,4), Richard E. Randall (2), Juan Ortín (1,4)
1 Department of Molecular and Cellular Biology, Centro Nacional de Biotecnología (CSIC), Darwin 3, 28049 Madrid, Spain.
2 School of Biology, Centre for Biomolecular Sciences, BMS Building, North Haugh, University of St Andrews, St Andrews, Fife KY16 9ST, UK
3 Laboratory Microbial Pathogenesis, Fundació d'Investigació Sanitària de les Illes Balears (FISIB), Recinto Hospital Joan March, Bunyola, Spain.
4 Ciber de Enfermedades Respiratorias, ISCIII.

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.

195 **Characterizing the antiviral response of the Pteropus vampyrus bat, an important ecological reservoir of henipaviruses**

Nicole B Glennon1,2, Omar Jabado3, Michael K Lo4, Megan L Shaw1
Department of Microbiology1, Graduate School of Biomedical Sciences2, Department of Genetics and Genomic Sciences3, Icahn School of Medicine at Mount Sinai, New York NY, USA and Centers for Disease Control & Prevention, Viral Special Pathogens Branch, Atlanta, Georgia, USA4

Increasingly, bats are being discovered to be important reservoirs for many viruses including, henipaviruses, SARS coronaviruses, ebola virus, and lyssaviruses. Despite being infected with a wide variety of viruses, most infections are reported to be asymptomatic in bats. Little is known about the bat antiviral response; therefore, we set out to characterize the innate immune response of the P. vampyrus bat. These bats are one of the reservoirs of Nipah virus (NiV) and Hendra virus (HeV), two paramyxoviruses that can infect humans with a high mortality rate. We obtained kidney tissue from a P. vampyrus bat, from which we prepared primary and immortalized P. vampyrus kidney (PVK) cells, and which we show support replication of NiV and HeV. We characterized the activation of the innate immune response in these cells upon infection with Newcastle disease virus (NDV), an avian paramyxovirus known to elicit a strong innate immune response in mammalian cells. We show that these cells induce interferon (IFN) in response to virus, but that this cytokine response has species-specific antiviral activity. Using mRNAseq technology we determined the transcriptome of mock- and NDV-infected PVK cells and identified host genes that are preferentially expressed in response to virus infection. In addition to known IFN-stimulated genes we detected some novel genes not previously associated with the innate immune response in other mammals. We confirmed a subset of these genes by RT-PCR and determined the induction kinetics of the antiviral response in PVK cells to NDV, NiV and HeV infection.

196 **Rabies virus P-protein interaction with STAT proteins is critical to lethal rabies Disease**

L. Wiltzer (1,2), S. Yamaoka (3), F. Larrous (4,5), H.V. Kuusisto (2), D. Blondel (6), A. Brice (1), H. Bourhy (4), D.A. Jans(2), N. Ito (3) and G.W. Moseley (1)
(1) Viral Pathogenesis Laboratory and (2) Nuclear Signalling Laboratory, Department of Biochemistry and Molecular Biology, Monash University, Victoria 3800, Australia.
(3) Laboratory of Zoonotic Diseases, Faculty of Applied Biological Sciences, Gifu University, Gifu, 501-1193, Japan.
(4) Institut Pasteur, Unit Lyssavirus Dynamics and Host Adaptation, Paris, France.
(5) Université Paris Diderot, Sorbonne Paris Cité, Cellule Pasteur, Rue du docteur Roux, 75015 Paris, France.
(6) Laboratoire de Virologie Moléculaire et Structurale, CNRS, Gif sur Yvette, France.

Lyssaviruses, including rabies virus, are a globally distributed genus of zoonotic viruses that cause rabies disease with a remarkable case-fatality rate of 100% in humans, resulting in >55,000 deaths/year worldwide. We and others have shown that lyssavirus P-proteins interact with transcription factors STAT1 and STAT2, which is thought to effect viral evasion of interferon (IFN)-mediated host innate immunity as a major factor in the development of disease. However, the genuine significance of this interaction during infection *in vivo* is unknown. Using an approach including multiple sequence alignments, site-directed mutagenesis, single cell imaging, immune signalling assays, protein-protein interaction analysis, viral reverse genetics, and an in vivo disease model we have identified the site mediating P-protein-STAT1/2 interaction for the first time, and have thus developed mutations that can specifically impair P-protein's function in inhibiting IFN/STAT responses. Importantly, the mutations do not affect genome replication enabling us to generate the first viable "STAT-blind" mutant lyssavirus. Growth in IFN-deficient cells is indistinguishable from parental wild-type virus, but is highly sensitive to IFN-treatment. Intracerebral inoculation of mice showed that the STAT-blind virus is profoundly attenuated *in vivo*, causing no neurological symptoms or fatalities, in stark contrast to the invariably lethal parental strain. This data provides the first direct evidence that P-protein-STAT1/2 interactions are critical to the development of lethal rabies, with clear significance to efforts to generate live attenuated vaccine strains and/or novel antiviral therapeutics to combat this incurable disease.

197 **Determinants for dsRNA-binding and PKR inhibition in the NS1 protein of influenza A virus as revealed by reverse genetics**

Kristina L. Schierhorn1, Katrin Hoegner2, Julia Dzieciolowski3, Susanne Herold2, Stephan Pleschka3, Thorsten Wolff1
1Robert Koch-Institute, Influenza and respiratory Viruses, Berlin, Germany; 2Justus-Liebig-University Gießen, Department for Internal Medicine II, Section of Infectious Diseases, Gießen, Germany;
3Justus-Liebig-University Gießen, Institute for Medical Virology, Gießen, Germany

The non-structural protein 1 (NS1) of influenza viruses functions in inhibiting the type I IFN-mediated antiviral state in infected cells. While NS1 proteins of influenza A and B viruses (A/NS1 and B/NS1) have only 20% sequence identity, they have a conserved N-terminal dsRNA-binding domain (RBD) and share functions as inhibition of the antiviral kinase PKR. We have previously shown that distinct basic amino acids in the RBD of B/NS1 are required for dsRNA-binding and inhibition of PKR. In contrast, A/NS1 has been suggested to silence PKR by a physical interaction involving a region outside the RBD. To evaluate whether the two influenza virus types in fact inhibit PKR by different means, we conducted a systematic analysis of the RBD of A/NS1 using reverse genetics. Among a panel of constructed mutants we identified 3 NS1 proteins with single basic amino acid exchanges eliminating dsRNA-binding. Two of the corresponding mutant viruses showed a 10-fold reduction in viral replication, possibly due to strong IFNβ induction. The third mutant virus was severely attenuated for replication by 4 logs, which was paralleled by strong activation of PKR, but very low IFNβ secretion. Significantly, replication of this mutant virus was largely rescued on PKR knock-down cells illustrating the strong impact of PKR on viral propagation. Thus, this study highlights a crucial role of the RBD of A/NS1 for PKR inhibition and viral replication. Moreover, we suggest that PKR has a major role in restricting influenza virus propagation among the more than 400 known type I IFN-stimulated factors.

198 **ISG15 and its role in the restriction of influenza A virus host range**

Jessica Knepper, Viola K. Weinheimer, Thorsten Wolff
Robert Koch Institute, Division 17 Influenza and Other Respiratory Viruses, Berlin

Influenza A virus (IAV) infection provokes an antiviral immune response including activation of IFN and IFN-stimulated proteins. One of these proteins is ISG15, which is a ubiquitin-like polypeptide that can be covalently attached to target proteins. The aim of this study was to elucidate the impact of ISG15 on the propagation of avian and human IAV in human cells. Our initial findings revealed strong induction of ISG15 in A549 cell cultures upon infection with seasonal IAV. We observed on single cell level that this induction occurred predominantly in uninfected cells, whereas little ISG15 was detected in the initially infected cells. A significantly increased number of both infected and ISG15-positive cells was observed after infection with a ΔNS1 mutant virus indicating a role for NS1 in suppressing ISG15 expression in the infected cells. Similarly, the number of double-positive cells was also increased upon infections with low-pathogenic avian IAV. Transfection-based experiments demonstrated that NS1 proteins of seasonal IAV significantly reduced subsequent IFN-stimulated ISG15-induction whereas this was not seen for NS1 proteins of low-pathogenic avian IAV. Interestingly, knockdown of ISG15 resulted in enhanced replication of low-pathogenic IAV in human cells, but did not affect propagation of seasonal IAV. In conclusion, our study reveals on single cell level the suppression of ISG15-induction by seasonal but not by low-pathogenic avian IAV and identifies NS1 as key player in this suppression. Thus, we hypothesize that ISG15 plays a vital role in IAV host range restriction and that seasonal IAV are able to counteract this antiviral response.

199 **IMPACT OF BORNA DISEASE VIRUS PERSISTENCE ON NEURONAL HOMEOSTASIS**

Alexandre Bétourné, Marion Szelechowski, Emilie Bonnaud, Anne Thouard, Charlotte Foret, Daniel Gonzalez-Dunia, Cécile E. Malnou
INSERM U1043, Centre de Physiopathologie de Toulouse Purpan, Paul Sabatier University, Toulouse, France

Borna disease virus (BDV) persistence in neurons causes central nervous disturbances characterized by behavioral and cognitive impairment. The exquisite BDV tropism for neurons is accompanied by selective interference with signaling pathways that are instrumental for the proper regulation of neuronal functioning. Although we and others have previously described some of the molecular pathways that are targeted by BDV, much remains to be learned on the precise mechanisms and consequences on neuronal activity and behavior. Recently, we have chosen two novel approaches to further investigate these aspects. In a first series of experiments, we have pursued our initial demonstration that BDV phosphoprotein (P) serves as a protein kinase C (PKC) decoy substrate when expressed in neurons, resulting in impaired neuronal activity. We have analyzed the impact of isolated expression of the P protein in vivo, in selected brain areas. This analysis revealed both PKC-dependent and independent effects of P on behavior. Incidentally, this analysis also revealed other consequences of the isolated expression of BDV components on neuronal survival and/or activity, such as our recent demonstration of the neuroprotective promise of BDV X protein. Finally, we have performed a detailed analysis of BDV impact on neuronal epigenetics. Indeed, epigenetic modifications are emerging as one of the key regulators of neuronal functioning. We will present our recent data demonstrating selective targeting of histone acetylation pathways resulting from BDV infection, as well as investigating the underlying molecular mechanisms and impact on neuronal function.

200 **Cedar virus: A novel henipavirus isolated from Australian bats**

Glenn A Marsh, Lin-Fa Wang
1 and 2 - Australian Animal Health Laboratory, CSIRO, Geelong, Australia
2 - Program in Emerging Infectious Diseases, Duke-NUS Graduate Medical School, Singapore

The henipaviruses, Hendra virus (HeV) and Nipah virus (NiV) causes serious and commonly lethal infection of people as well as various species of domestic animals. Pteropid bats have been shown to act as the main natural reservoir for both of these viruses. In a recent study, investigating HeV prevelance in bat colonies, we isolated a new paramyxovirus, Cedar virus (CedPV), which shares significant features with the known henipaviruses. The genome size (18,162 nt) and organization of CedPV is very similar to that of HeV and NiV; its nucleocapsid protein displays antigenic cross-reactivity with henipaviruses; and it uses the same receptor molecule (ephrin- B2) for entry during infection. Preliminary challenge studies with CedPV in ferrets and guinea pigs, both susceptible to infection and disease with known henipaviruses, demonstrated virus replication in the absence of clinical disease. Sequence comparisons demonstrated that the major genetic difference between CedPV and HeV or NiV lies within the coding strategy of the P gene, which is known to play an important role in evading the host innate immune system. Unlike HeV, NiV, and almost all known paramyxoviruses, the CedPV P gene lacks both RNA editing and also the coding capacity for the highly conserved V protein. Preliminary study indicated that CedPV infection of human cells induces a more robust IFN-b response than HeV. The discovery of a non-pathogenic henipavirus will provide a powerful tool for us to better understand the pathogenesis of HeV and NiV, which may lead to novel approaches for development of therapeutics.

201 **Soluble Proteins Of Ebola Virus Activate Dendritic Cells And Macrophages Causing Release Of Pro- And Anti-Inflammatory Cytokines**

Beatriz Escudero Pérez, Philip Lawrence and Viktor Volchkov *CIRI, Inserm U1111, Lyon, France*

Ebola virus (EBOV) is a member of Filoviridae family and causes hemorrhagic fever with high fatality rates. Through RNA editing the EBOV GP gene codes for both the highly glycosylated surface protein (GP) and the secreted glycoprotein sGP. Surface GP is responsible for virus attachment and membrane fusion, but is also released from cells in a soluble form (shed GP) due to cleavage by cellular metalloprotease TACE.

Both dendritic cells (DCs) and macrophages serve as early targets of EBOV and play a prominent role during infection. No cellular target has yet been identified for EBOV soluble proteins. Here, for the first time we demonstrate that EBOV soluble proteins bind to human monocyte-derived DCs and macrophages. Importantly, we demonstrate that binding of shed GP but not sGP causes an activation of CD40, CD80 and CD86 expression and an increase in levels of mRNA encoding TNFα, IL6, IL10 and IL12p40. Treatment of DCs and macrophages with shed GP resulted in release of both pro- and anti-inflammatory cytokines, as demonstrated by multiplex ELISA. We have also revealed that the glycosylation pattern of shed GP is crucial in mediating this activation. Overall, this study suggests that shed GP but not sGP is responsible for the early stimulation of human DCs and macrophages and thus may play a role in the dysregulation of host immune responses that, combined with massive virus replication and virus-induced cell damage, leads to a septic shock-like syndrome and high mortality.

202

Human Metapneumovirus SH and G glycoproteins Inhibit Macropinocytosis-Mediated Entry Into Dendritic Cells and Reduce CD4+ T Cell

Cyril Le Nouën 1, Philippa Hillyer 2, Linda G Brock 1, Christine C Winter 1, Ronald L Rabin 2, Peter L Collins 1, and Ursula J Buchholz 1.
1 RNA Viruses Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 2 Center for Biologics Evaluation and Research, US Food and Drug Administration.

Human metapneumovirus (HMPV) is a major etiologic agent of respiratory disease worldwide. HMPV reinfections are common in healthy adults and children, suggesting that the protective immune response to HMPV is incomplete and short-lived. Deletion of the attachment G and small hydrophobic SH glycoproteins (ΔSHG) increased infectivity of primary human monocyte-derived dendritic cells (MDDC) in vitro but had little effect on MDDC maturation. However, MDDC stimulated with ΔSHG induced increased proliferation of autologous Th1-polarized CD4+ T cells. This effect was independent of viral replication. This increased T cell proliferation was strictly dependent on contact between the virus-stimulated MDDC and the CD4+ T cells, and MDDC stimulated with UV-inactivated ΔSHG established more immunological synapses with memory CD4+ T cells compared to UV-inactivated WT HMPV. Using time course experiments and a panel of inhibitors we found that uptake of HMPV by MDDC was mediated primarily by macropinocytosis. Uptake of WT virus was delayed and reduced compared to ΔSHG, indicative of inhibition by the glycoproteins. In addition, DC-SIGN-mediated endocytosis provided a minor alternative pathway that depended on SH and/or G and thus operated only for WT HMPV. Altogether our results show that SH and G glycoproteins reduce the ability of HMPV to be internalized by MDDC, resulting in a reduced ability of the HMPV-exposed MDDC to activate CD4+ T cells. This study describes a previously unknown mechanism of virus immune evasion.

203

Wild Type Measles Virus Infection Up-Regulates PVRL4 and Causes Apoptosis in Brain Endothelial Cells by Induction of TRAIL

Hani'ah, Abdullah 1, Brenda Brankin 2, Clare Brady 1, S. Louise Cosby
1. Centre for Infection and Immunity, School of Medicine Dentistry and Biomedical Sciences, Queen's University Belfast, UK
2. School of Biological Sciences, Dublin Institute of Technology, Dublin, Ireland

In the long term measles virus (MV) complications subacute sclerosing panencephalitis (SSPE) and measles inclusion body encephalitis brain endothelial cell (BEC) infection is likely to play a major role in virus spread into surrounding brain tissue facilitated by damage to the blood brain barrier. We previously found in SSPE brain that BEC in some blood vessels are infected but the entry receptor used is unknown. WT viruses do not use CD46 and BEC are SLAM negative. It is not known if these cells express the MV epithelial receptor PVRL4 (Nectin 4). We infected human (H) and murine (M) BEC and mice with WT, vaccine and rodent adapted MV strains to elucidate events in the cerebral endothelium. While uninfected HBEC were negative for PVRL4, this molecule was expressed in areas of virus infection. Although efficient WT MV production occurred in MBEC, infected cells were PVRL4 negative. Extensive monolayer destruction associated with activated caspase 3 staining was observed in both HBEC and MBEC, most markedly with WT MV. Tumour necrosis factor related apoptosis inducing ligand (TRAIL), but not FAS ligand, was induced by MV infection. Treatment of virally induced supernatants with anti-TRAIL antibody successfully blocked caspase 3 expression and monolayer destruction. TRAIL was expressed primarily in the endothelium and in other cell types in infected murine brain. This is the first demonstration that WT MV infection of BEC induces TRAIL in vitro and in vivo with subsequent apoptosis. We also report the novel finding that infection of HBEC up-regulates expression of PVRL4.

204 **Furin cleavage and in vivo imaging of Crimean-Congo hemorrhagic fever virus secreted non-structural glycoproteins**

Éric Bergeron1, Dennis A. Bente2, Ayan K. Chakrabarti1, Alexander McAuley2, Jessica R. Spengler1, Stuart T. Nichol1, César G. Albariño1, Christina F. Spiropoulou1 *Centers For Disease Control and Prevention1, University of Texas Medical Branch2*

Crimean-Congo hemorrhagic fever virus (CCHFV) is a tick-borne RNA virus (family Bunyaviridae, genus Nairovirus) that causes lethal hemorrhagic fever in humans. Cloning difficulties of the unusually long L segment (~12 kb) and need for biosafety level 4 containment have hampered the recovery of recombinant CCHFV (rCCHFV). We have produced rCCHFV entirely from DNA for the first time. Viral glycoprotein Gn is derived from cleaving a long Gn precursor (PreGn) comprised of a mucin-like domain (MLD), a GP38, and a Gn domain. The Golgi resident site-1 protease cleaves PreGn between GP38 and Gn, leading to incorporation of Gn into nascent virions and secretion of non-structural glycoproteins (NSGs). NSG cleavage by furin detaches MLD from GP38 into the trans-Golgi network. To measure the role of furin in NSG processing during replication, we obtained rCCHFV resistant to furin cleavage (Δfur). Using Δfur, we demonstrate that furin enhances CCHFV replication by cleaving the canonical RSKR motif located between MLD and GP38. In addition, furin cleavage of unknown host substrate(s) or of other sites within viral glycoproteins are required for efficient viral propagation. To visualize CCHFV infection and explore the possible functions of NSGs, we fused mCherry to MLD or GP38 to obtain rCCHFV producing red fluorescence in vitro and in mice. Viral growth kinetics and disease progression of mCherry-CCHFV were comparable to wild type. In contrast to rCCHFV, mCherry-CCHFV-infected mice recovered from infection. Novel rescue system and mCherry-CCHFV are valuable tools to study the immune response, test therapeutics, and characterize NSGs role in pathogenesis.

205 **Contribution of the NSm proteins of Rift Valley Fever virus to virus propagation and virulence in mammalian and arthropod hosts**

Felix Kreher 1,2, Carole Tamietti 1,2, Celine Gommet 3, Laurent Guillemot 3, Anna-Bella Failloux 1,4, Jean Jacques Panthier 3, Michèle Bouloy 1, Marie Flamand 1,2
1. Moleculare Biology of Bunyaviruses, Institut Pasteur, Paris, France
2. Structural Virology, Institut Pasteur, Paris, France
3. Mouse Functional Genetics, Institut Pasteur, Paris, France
4. Arboviruses and Insect Vectors, Institut Pasteur, Paris, France

Rift valley fever is a zoonotic disease in Africa primarily affecting livestock and humans. Rift valley fever virus (RVFV) is an enveloped segmented RNA virus. The viral genome is divided into three segments, L, M and S, in negative or ambisense polarity. The M segment encodes the two major structural glycoproteins, GN and GC, and at least two accessory proteins, P78/NSm-GN and P14/NSm, through the alternate use of 5 different in-frame AUG codons. We analyzed the impact of the different NSm-related proteins on RVFV propagation in vitro and in vivo. Specific AUG translation initiation sites were modified and RVFV mutants expressing different sets of the NSm-related proteins were reconstituted in vitro using a reverse genetic system. Infectivity of the different mutant viruses was compared to a rescued wt virus in a mouse model of intraperitoneal infection or in blood-fed mosquitoes. None of the NSm-related proteins deleted individually had any significant effect in the mouse model. Only the combined knock-out of P14/NSm and P13/NSm', a N-terminally truncated form of P14/NSm, led to a drastic reduction in virulence. Interestingly, P78/NSm-GN appeared as a major determinant of virus dissemination in the infected mosquitoes. This study points to different contributions of the NSm-related proteins to virus propagation in the mammalian and arthropod hosts, the NSm/NSm' proteins being essential for viral virulence in mice and the P78/NSm-GN glycoprotein conditioning virus spread in the mosquito vector. The role of the NSm-related proteins in the RVFV life cycle will be further discussed in the context of the different in vitro and in vivo models.

206 **Hantavirus infection confers resistance to NK cell-mediated killing and hantavirus N protein inhibits gzmB and caspase 3**

Shawon Gupta1,2, Monika Braun3, Nicole Tischler4, Malin Stoltz2, Karin Sundström1,2, Niklas Björkström3,5, Hans-Gustaf Ljunggren3 and Jonas Klingström1,2,3
1 Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, 171 77 Stockholm, Sweden
2 Department of Preparedness, Swedish Institute for Communicable Disease Control, 171 82 Solna, Sweden
3 Center for Infectious Medicine, Department of Medicine, Karolinska Institutet, Karolinska University Hospital Huddinge, 141 86 Stockholm, Sweden
4 Fundación Ciencia & Vida, 778 0272 Santiago, Chile
5 Liver Immunology Laboratory, Division of Gastroenterology and Hepatology, Department of Medicine, Karolinska Institutet, Karolinska University Hospital Huddinge, 141 86 Stockholm, Sweden

Hantaviruses cause hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardio-pulmonary syndrome (HCPS), two human diseases with high case fatality rates. Endothelial cells are the main targets for hantaviruses and vascular permeability is a hallmark of HFRS/HCPS. An intriguing observation in patients with HFRS/HCPS is that the virus infection leads to strong activation of cytotoxic lymphocytes, CD8 T cells and Natural Killer cells, but no obvious destruction of infected endothelial cells. Here, we provide a possible explanation for this dichotomy by showing that hantavirus-infected endothelial cells are protected from cytotoxic lymphocyte-mediated killing. Hantaviruses were also able to inhibit chemically induced apoptosis in both endothelial and epithelial cells. When dissecting potential mechanisms behind this phenomenon, we discovered that the hantavirus nucleocapsid (N) protein contains multiple granzyme B sites and at least one caspase 3 site and that the N-protein inhibits the enzymatic activity of both granzyme B and caspase 3. Transfection experiments showed that the N-protein by itself was able to inhibit apoptosis. In a closer evaluation of the inhibitory caspase 3 site in the N-protein we could conclude that the site is DLID285. Alanin replacements of DLID285 to DLIA285 rendered N-protein transfected cells susceptible to apoptotic stimuli. Our findings provide a tentative explanation for the hantavirus-mediated block of cytotoxic granule-mediated killing, and hence the protection of infected cells from cytotoxic lymphocytes. These findings may explain why infected endothelial cells in hantavirus-infected patients are not destroyed by the strong cytotoxic lymphocyte response.

207 **The hemagglutinin, nucleoprotein and neuraminidase gene segments contribute to virulence in a chimeric H4/H5 avian influenza vir**

Jutta Veits, Jana Hundt, Siegfried Weber, Jürgen Stech and Thomas C. Mettenleiter
Institute of Molecular Biology, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, 17493 Greifswald – Insel Riems, Germany

Avian influenza viruses (AIV) possess segmented, negative-sense RNA genomes and belong to the family Orthomyxoviridae. Due to their virulence AIV are classified as low pathogenic (LP), exhibiting hemagglutinins (HA) of all known subtypes (H1-H16), or highly pathogenic (HP). HPAIV are restricted to the subtypes H5 and H7 with the main virulence determinant of a polybasic cleavage site (PCS) within the HA, which enables a proteolytic activation by ubiquitous proteases and in contrast to LPAIV are therefore capable to cause severe systemic disease in poultry. To investigate if this feature is sufficient to shift an LP to an HP phenotype in a non-H5/H7 background, we cloned A/mallard/Germany/1240/1/07 (H4N6) by reverse genetics with an artificial PCS (H4N6hp). However, the introduction of a PCS caused only a slight increase of virulence in infected chickens. Therefore, to investigate additional virulence determinants beside the PCS, we generated chimeric AIV of H4N6hp with all eight single gene segments of a H5N1 HPAIV. Reassortants exhibiting the H5N1 segments HA, neuraminidase (NA) and nucleoprotein (NP) showed increased virulence in chickens in the potency of HA, NP and NA. In concordance, reassortants with all possible combinations of these three segments in H4N6hp increased the virulence further on with lethal outcome. Therefore, besides the hemagglutinin and neuraminidase the nucleoprotein of H5N1 reveals a potent virulence determinant, at least in the given H4 background.

208 **Mitochondrial Targeting Sequence Mutations In PB1-F2 Protein Enhance The Virulence of Pandemic H1N1 Influenza A Virus**

Subbiah Elankumaran, Jagadeeswaran Deventhiran, Sandeep RP Kumar *Biomedical Sciences and Pathobiology, VA-MD Regional College of Veterinary Medicine Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA*

Influenza A virus (IAV) PB1-F2 protein, is considered to play an important role in virulence. The 2009 H1N1 pandemic (pdm) IAV was highly transmissible but was mild clinically. The lack of expression of PB1-F2 was speculated, but when PB1-F2 expression was reconstituted, it had minimal impact on virulence to mice and pigs. The mitochondrial-targeting sequence (MTS) of PB1-F2 dictates its pro-apoptotic and pro-inflammatory functions. The presence of serine at position 66 of the MTS (66S), previously shown to enhance virulence in mice, increased only in vitro virus replication of pdm IAV. Other genetic mutations as seen in past pandemic viruses could occur in nature or derived through reassortment in pdm IAV. We have addressed this by creating 66S and 66S+2, 66S+3, 66S+5 mutations in reconstituted PB1-F2 protein of pdm IAV. All MTS mutant viruses grew to higher titers and produced larger plaques than knock-in (KI) or wild type (wt) viruses in vitro. In Balb/c mice, MTS mutants except 66S+5 induced severe clinical disease and significant morbidity and mortality compared to KI and wt viruses. An increased influx of macrophages and neutrophils and altered protein levels of pro-inflammatory cytokines were noticed in the lungs of mice infected with 66S+2 and 66S+3 mutants. Microscopically, enhanced bronchointerstitial pneumonia and lymphocytic infiltration were observed as early as 3 days post infection in sick mice. In summary, our study shows that specific amino acids in the MTS region of PB1-F2, in addition to 66S, determine the virulence of 2009 H1N1 pdm IAV.

209 **NEWLY IDENTIFIED MOTIFS OF INFLUENZA A PROTEIN PB1-F2 THAT PRIME VIRAL AND SECONDARY BACTERAL PNEUMONIA**

Irina Alymova,1 Amali Samarasinghe,3 Amanda Green,1 Peter Vogel,2 Jon McCullers 1,3 *Department of 1Infectious Diseases and 2Animal Research Center, St. Jude Children's Research Hospital, Memphis, TN, USA; 3Department of Pediatrics, University of Tennessee Health Sciences Center, Memphis, TN, USA.*

The influenza A protein PB1-F2 possesses two functionally distinct motifs capable of promoting viral and secondary bacterial pneumonia. The L62, R75, R79, and L82 motifs of A/Hong Kong/1/68 PB1-F2 facilitated inflammatory activity. The I68, L69, and V70 motifs of A/Puerto Rico/1/34 PB1-F2 induced permeabilization of mitochondrial membranes and death in 70% cells. In the mouse model, the presence of either domain in PB1-F2 enhanced virus replication, increased acute lung injury and levels of pro-inflammatory cytokines compared to infection with mutant viruses lacking proper motif. In the mouse secondary Streptococcus pneumoniae model, the presence of either domain in the influenza A PB1-F2 increased viral and bacterial lung titers (at days 1, 2, and 3 after bacterial challenge), and mortality (up to 70%). The associated loss of alveolar epithelium integrity significantly contributed to the extensive lung damage caused by PB1-F2 "cell death" motif in the secondary bacterial infection model. These data suggest that newly identified sequences of influenza A protein PB1-F2 contribute to the pathogenesis of both primary viral and secondary bacterial infections. The inflammatory motif was found in all three of the 20th century pandemic strains as well as representatives of highly pathogenic avian influenza viruses of the H5N1 subtype and many currently circulating swine viruses. The "cell death" domain is present in the PB1-F2 proteins of seasonal H1N1 and H3N2 influenza A viruses. These specific residues of PB1-F2 could be utilized in pandemic planning as molecular signatures of the predicted severity of primary viral and secondary bacterial infections.

210 **A Systems Biology Approach Reveals Novel Host Response Genes that Differentially Regulate Influenza Virus Pathogenicity**

Amie J. Eisfeld (1), Hugh Mitchell (2), Michael G. Katze (3), Katrina M. Waters (2), and Yoshihiro Kawaoka (1)
1-Influenza Research Institute, Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI; 2-Computational Biology and Bioinformatics Group, Pacific Northwest National Laboratory, Richland, WA; 3-Department of Microbiology, School of Medicine, University of Washington, Seattle, WA

Influenza viruses can cause severe disease in humans, and while the mechanisms are incompletely understood, host response dysregulation is thought to contribute. To uncover novel host factors that regulate influenza virus pathogenicity, we used a systems biology approach comprising virus infections in mice, global transcriptome analysis of infected lung tissues at multiple time points after infection, and application of a computational algorithm (weighted gene correlation network analysis, 'WGCNA'). WGCNA identifies transcripts exhibiting highly connected (i.e., 'hub-like') expression behavior, which suggests a regulatory influence over the host transcriptional response. To assess the role of hub-like transcripts in influenza virus pathogenesis, we compared virus-induced disease in wild-type mice and knockout (KO) mice lacking expression of specific hub-like genes (Ripk3 and Saa1) following infection with influenza A/California/04/2009 (H1N1), a 2009 pandemic virus. Ripk3 KO mice exhibited less severe disease, significantly reduced weight loss and faster recovery compared to wild-type mice, implying that Ripk3 activity is detrimental for the outcome of infection. In contrast, Saa1/Saa2 double knockout mice exhibited more severe disease, increased weight loss and reduced survival, suggesting a protective role. These results illuminate novel host genes that differentially regulate influenza virus pathogenicity, and lay the foundation for future studies aimed at clarifying the mechanisms of influenza virus-induced disease and the discovery of new treatment strategies. This project was funded in whole or in part with federal funds from the NIAID, NIH and DHHS under contract #HHSN272200800060C.

211 **Efficient induction of viral encephalitis after aerosol exposure to Rift Valley Fever Virus**

Amy L. Hartman(1,2), Diana S. Powell(1), Laura M. Bethel(1), Amy L. Caroline(1,2), Jacquelyn M. Bales(1), Richard J. Schmid (3), Timothy D. Oury (3), and Douglas S. Reed (1,4)
1 - Regional Biocontainment Laboratory, University of Pittsburgh Center for Vaccine Research
2 - Department of Infectious Diseases and Microbiology, University of Pittsburgh Graduate School of Public Health
3 - Department of Pathology, University of Pittsburgh School of Medicine
4 - Department of Immunology, University of Pittsburgh School of Medicine

Rift Valley Fever virus (RVFV) is an arbovirus that causes significant morbidity and mortality in both humans and livestock. Humans infected with Rift Valley Fever virus can develop severe complications including acute hepatitis, fatal hemorrhagic fever, ocular disease, and delayed-onset encephalitis. Inbred rat strains differ in their susceptibility and disease course after parenteral or aerosol infection with RVFV. ACI rats develop encephalitis after either s.c. inoculation or inhalation of RVFV. In contrast, Lewis rats are highly resistant to RVFV by s.c. inoculation but develop fatal encephalitis after inhaling small doses of RVFV. In both strains, lethality is increased and the time to death is shortened with aerosol infection compared to s.c. inoculation. Serial sacrifice studies show that after aerosol exposure, ACI and Lewis rats have low levels of virus replication in tissues outside the lung until the last 2 days when high levels of virus are found in the brain. The mechanism behind the increased incidence of neurological disease after aerosol exposure to RVFV is under investigation. In both rat strains, a pronounced leukocytosis was seen in the blood over the course of the infection, the bulk of which was neutrophils and other granulocytes (>10-fold increase in total granulocyte counts for both ACI and Lewis rats). Pathological evaluation found evidence of meningoencephalitis in the brains of both ACI and Lewis rats. These data suggest that both strains of rat can serve as appropriate tools to understand the pathogenesis of neurological disease resulting from infection with Rift Valley Fever virus. ** This is a U.S. Department of Defense's Joint Program Executive Office-Chemical Biological Defense (JPEO-CBD) program supported by the Joint Project Manager Transformational Medical Technologies (JPM-TMT) program through the Defense Threat Reduction Agency (DTRA) contract HDTRA1-10-C-1066.

212 **Heterologous exchanges of non-virion protein and glycoprotein in Novirhabdoviruses:effects on pathogenicity and host specificity**

Arun Ammayappan1, Gael Kurath2, Tarin Thompson2, Shamila Yusuff1, Vikram Vakharia1 *Institute of Marine & Environmental Technology, University of Maryland Baltimore County, 701 East Pratt St., Baltimore, MD 21202, USA1, US Geological Survey, Western Fisheries Research Center, Seattle, WA 98115*, USA2

Infectious hematopoietic necrosis virus (IHNV) and viral hemorrhagic septicemia virus (VHSV) are rhabdovirus species belonging to the Novirhabdovirus genus. IHNV has a narrow host range restricted to trout and salmon species, and it does not infect yellow perch. In contrast, the VHSV genotype IVb that invaded the Great Lakes in United States has a broad host range, with high virulence in yellow perch, but not in trout. By using reverse-genetic systems of IHNV and VHSV, we generated six IHNV:VHSV chimeric viruses in which the glycoprotein (G), non-virion-protein (NV), or both G and NV genes of IHNV-M genotype were replaced with the analogous genes from the VHSV-IVb, and vice versa. Viable viruses were recovered in all cases and used to challenge groups of rainbow trout and yellow perch. The parental recombinants rIHNV-M and rVHSV-IVb were virulent in trout and yellow perch, respectively. In yellow perch, chimeric IHNV viruses with substitutions of the G, NV and G +NV of VHSV were not virulent, whereas in trout, virus with the NV substitution was avirulent; virus with the G substitution was virulent, and virus with G+NV substitutions was partially virulent. In reciprocal swaps, chimeric VHSV-IVb viruses with the G, NV and G +NV substitutions of IHNV were avirulent in trout, whereas in yellow perch, only the chimeric virus with NV substitution was virulent. These results suggest that the G and/or NV genes are not, by themselves or in combination, sufficient to determine host-specific virulence.

213 **Experimental infection of rhesus and cynomolgus macaques with a wild water bird-derived highly pathogenic avian influenza virus**

Tomoko Fujiyuki1, Misako Yoneda1, Fumihiko Yasui2, Takeshi Kuraishi3, Shosaku Hattori3, Hyun-jeong Kwon1, Keisuike Munekata2, Yuri Kiso1, Hiroshi Kida4, Michinori Kohara2, Chieko Kai1
1.Laboratory Animal Research Center, The Institute of Medical Science, The University of Tokyo,
2.Department of Microbiology and Cell Biology, Tokyo Metropolitan Institute of Medical Science
3.Amami Laboratory of Injurious Animals, The Institute of Medical Science, The University of Tokyo,
4.Graduate School of Veterinary Medicine, Hokkaido University

Highly pathogenic avian influenza virus (HPAIV) continues to threaten human health. Non-human primate infection models of human influenza are desired. However, there have only been a few studies of HPAIV infection in rhesus macaques. To establish an animal infection model with more natural transmission and to determine HPAIV pathogenicity in primates, we investigated whether droplet exposure of macaques to a Japanese isolate of HPAIV (A/whooper swan/Hokkaido/1/2008, H5N1 clade 2.3.2.1) could induce influenza symptoms using two different macaque species, rhesus and cynomolgus monkeys, with the aim of understanding the virulence of this strain in non-human primates, the efficacy of droplet exposure for infection, and common or different properties of macaque species in terms of HPAIV pathogenicity. We administered this HPAIV to rhesus and cynomolgus monkeys via multipleroutes including the intratracheal and nasal routes with droplet exposure. Infection was observed in the lower and upper respiratory tracts of macaques. Viral distribution in the respiratory tract was broader and viral shedding was more obvious in cynomolgus monkeys than in rhesus macaques. Inoculation with higher doses of the isolate resulted in stronger clinical symptoms of influenza. Our results demonstrate that HPAIV isolated from a water bird in Japan is pathogenic in monkeys by experimental inoculation, and a new method for HPAIV infection of non-human primate hosts, a good animal model for investigation of HPAIV pathogenicity for development of vaccines and therapeutics.

214 **Symptomatic highly pathogenic avian influenza virus restores pathogenicity in chicken by serial egg pasajes**

Yuko Uchida, Yasushi Suzuki, Takehiko Saito

Existence of multiple basic amino acids at the cleavage site of hemagglutinin (HA) of H5 and H7 subtypes influenza viruses is attributed to pathogenicity of highly pathogenic avian influenza virus (HPAIV) in chickens. To explore other genetic signatures contributing to the pathogenicity in chickens, several recombinant influenza viruses were generated by reverse genetics and infected to chickens. These viruses possessed HA and neuraminidase (NA) segments from an H5N1 HPAIV and internal gene segments from two low pathogenic AIVs. One of the recombinant viruses, designated as LP (W/PA), did not kill chickens at all by intranasal and intravenous infection although retaining multiple basic amino acids at the cleavage site. LP (W/PA) was passaged 16 times in 14-day-old embryonated eggs and viruses passaged once and 16 times were designated as LP (W/PA) E1 and LP (W/PA) E16, respectively. Each of 107EID50 virus was intravenously inoculated to chickens and intravenous pathogenicity index (IVPI) was determined. The LP (W/PA) E16 killed all of chickens within 6 days post inoculation (dpi) whereas none of chickens infected with LP (W/PA) E1 died during 10 days of observation period. LP (W/PA) E16 was determined as HPAIV as IVPI was greater than 1.2 based on the OIE manual. Amino acid substitutions of LP (W/PA) E16, compared to LP (W/PA) E1, were found in HA and PA segments. No substitution was observed in the cleavage site. Contribution of those substitutions to the pathogenicity in chicken requires further scrutiny.

215 **The incubation period is longer than the latent period in chickens and turkeys experimentally infected with Highly Pathogenic Av**

James Seekings, Chad Fuller, Caroline Warren, June Mynn, Ross Cooper, Elizabeth Aldous, Richard M. Irvine and Ian H. Brown *AHVLA – Weybridge*

In the European Union, and many other countries world-wide, control measures for highly pathogenic avian influenza (HPAI) are imposed by legislation and involve stamping out policies with defined restriction zones and severe trade implications. Early detection of outbreaks can minimise losses and cost through reducing potential spread. This work generated experimental data of first clinical signs in chickens and turkey's infected with HPAI and the relationship between the incubation period and latent period. Ten 3-week-old chickens and turkeys were infected with either H7N1 or H5N1 HPAI, using a dose 0.5 log10 above the previously determined MLD50, via the intraocular and intra nasal route. All of the birds were inspected regularly and clinical signs observed and scored. Buccal and cloacal swabs were taken up to three times a day and tested using real time RT-PCR. Indices were calculated for clinical signs as well as buccal and cloacal virus shedding to allow comparison of the latency and incubation periods. For both H7 and H5 in chickens and turkeys the latent period was shorted than the incubation period indicating a period of subclinical infection of approximately 12 hours. The high risk period between infection and the onset of observed clinical signs was less than 28 hours with 100% mortality by 48 hours.

216 **Pathogenesis of infection of pregnant gilts with H1N2 swine influenza virus**

Iwona Markowska-Daniel, Krzysztof Kwit, Małgorzata Pomorska-Mól
Swine Diseases Department, National Veterinary Research Institute, Pulawy, Poland

Objectives In the course of influenza in pregnant females, respiratory or systemic infection is sometimes accompanied with abortion. The aim of the project was to study the pathogenesis of infection of pregnant gilts with swine influenza virus (SIV). Materials and methods), acute phase proteins (CRP, Hp) in serum and reproduction parameters were evaluated.gPregnant gilts were infected intranasally or intratracheally with H1N2 SIV. Clinical signs, presence of viral RNA in nasal swabs, placenta and lungs of piglets, HI titer, T-cell specific immunity, hematological parameters, concentration of selected interleukin (IL-10, IFN Results However no fever or any other clinical sings typical for SI were found in all infected gilts, the experimental infection was successful because specific antibodies against SIV were confirmed in all infected gilts from 14 dpi, antigen-specific proliferation from 6 dpi and the presence of SIV RNA in nasal swabs was detected from 1 to 4 dpi. Any of the infected gilts do not have any signs of pregnancy pathology. No significant differences were found between infected and control gilts with regard to hematological parameters, concentrations of APP and cytokines tested. No SIV RNA were found in samples taken from newborn piglets and placenta. Conclusion The results of this study indicate a lack of intrauterine transmission of SIV, H1N2 subtype, from mother to fetal. They tend to support the hypothesis that high fever and inflammation play a main role in the pathogenesis of abortion and other reproduction disorders in the course of the swine influenza.

217 **Novel paramyxovirus causes severe illness in a wildlife biologist working in South Sudan and Uganda**

Cesar G.Albariño (1), Michael Foltzer (2), Jonathan Towner(1), Mike Frace (3), Shelley Campbell (1), Carlos M. Jaramillo (2), Brian Bird (1), DeeAnn Reeder (4), Megan Vodzak (4), Paul Rota (5), Christina Spiropoulou (1), Barbara Knust (1), Stuart T. Nichol (1), Pierre E. Rollin (1), Ute Ströher (1)
(1)Viral Special Pathogens Branch, CDC, Atlanta GA,USA
(2)Geisinger Medical Center, Danville PA, USA
(3)Biotechnology Core Facility Branch,CDC, Atlanta GA,USA
(4)Bucknell University, Lewisburg PA, USA
(5)Measles, Mumps, Rubella & Herpes Virus Laboratory Branch,CDC, Atlanta GA,USA

Paramyxoviruses comprise a large group of viruses, including severe human pathogens. In recent years, over 100 new paramyxoviruses were identified in bats and rodents worldwide. Among those, very few are known to cause human disease, possibly because of the usually limited host range and rare crossover Nevents. Here, we describe a novel paramyxovirus that caused very severe disease in a wildlife biologist who participated in a 6-week field expedition to South Sudan and Uganda. During this expedition, she was exposed to more than 20 different bat and rodent species. She wore different levels of personal protective equipment during these encounters. Shortly after returning to the United States, she developed fever, malaise, headache, generalized myalgia and arthralgia, neck stiffness, and a sore throat. After admisión to the hospital, she developed a maculopapular rash that became confluent over time. Laboratory tests demonstrated thrombocytopenia and profound leukopenia, as well as elevated serum levels of liver aminotransferases. Molecular diagnostic testing was negative for filoviruses and selected bunya-, arenaand flaviviruses. Next-generation sequencing revealed the presence of a novel paramyxovirus. The complete sequence was obtained by standard RT-PCR and Sanger sequencing. Phylogenetic analysis of the genome indicated that the virus was most closely related to a rubula-like virus, Tuhoko virus 3, recently detected in *Rousettus leschenaultii* fruit bats in southern China. Pairwise comparison of the novel virus genome showed 66.2% identity to Tuhoko virus 3, while protein sequences were 54–84% identical.

218 **Type II pneumocytes are the major target cells for seasonal and highly pathogenic influenza A viruses in the human lung**

Viola K. Weinheimer 1, Anne Becher 2, Jessica Knepper 1, Stefan Hippenstiel 2, Andreas Hocke 2, Thorsten Wolff 1
1 Robert Koch-Institut, Div. of Influenza and other Respiratory Viruses, Berlin, Germany;
2 Dept. of Internal Medicine/Infectious Diseases and Pulmonary Medicine, Charité Universitaetsmedizin Berlin, Germany

Differences in the cellular tropism of closely related viruses may strongly affect their virulence. In case of highly pathogenic avian H5N1 influenza viruses it had been hypothesized that preferential infection of alveolar type II pneumocytes contributes to their high pathogenicity in humans thereby inhibiting repair processes in the lung and triggering dysregulated immune responses. Whether this is a valid possibility was uncertain as the primary target cells of seasonal and animal influenza viruses in human lung had not been systematically studied. Here, we employed a human lung organ culture model in which the complexity and spatial arrangement of cell types is preserved to study the replication, tropism and cytokine induction by human, avian and porcine influenza A viruses. The primary tissue supported efficient growth of highly-pathogenic H5N1 and human-adapted H1N1 and H3N2 viruses, whereas classical swine and low pathogenic avian viruses replicated only poorly. Nevertheless, all viruses examined were detected almost exclusively in type II pneumocytes with a minor involvement of alveolar macrophages. Infection with low and high pathogenic avian viruses provoked a pronounced induction of IP-10, MIP-1β, IFN-β and IL-1β, while human and pandemic H1N1-2009 viruses triggered a weak cytokine response. These findings show that differences in the pathogenic potential of influenza A viruses are unlikely to be determined by a distinct cell tropism in the human lung. Rather, high or low viral pathogenicity appears to be associated with a strain-specific capacity to replicate productively in type II pneumocytes and to cope with the induced cytokine response.

219 **Subacute Sclerosing Panencephalitis: Where does Measles Virus persist inside the host? Some facts and one hypothesis**

Rafael Fernandez-Muñoz1, Juan Carabaña*, Monserrat Caballero*, Miguel A. Muñoz-Alía 1,3, and María L. Celma1
1.Unidad de Virología, Hospital Ramón y Cajal, Madrid, Spain
**.Present address: Duke University Medical School, NC, U.S.A.*
3.Centro Nacional de Biotecnología ,C.S.I.C, Campus de Cantoblanco, Madrid, Spain.

The study of measles virus (MV) genotypes circulating consecutively in Madrid from 1960s to 1990s and MV genomes from Subacute Sclerosing Panencephalitis (SSPE) brain autopsies along that period previously allowed us to confirm the hypothesis that MV causing SSPE correspond with the one causing the acute infection. It remains unclear, however, where MV may persists after the acute infection. We sequenced the complete MV genome present in the brain from three SSPE patients who died after short (4 months), intermediate (4 years) and long disease course (18 years) . The genome length, was preserved in the 3 cases (15894; 15894, and 15893/15894 nucleotides). Selective RT-PCR amplification for MV copy-back MV RNA was negative in 2 cases and slightly positive in the third. On the other hand for the 3 patients brain nucleocapsid RNA hybridization with (P32) probes complementary to different regions along MV genome showed not significant presence of defective viral genomes. In addition, from 2 of SSPE patients we obtained thoracic and abdominal lymph nodes at autopsy. In both cases we amplified genomic MV RNA corresponding to N, M, and F genes from different lymph nodes. The MV in lymph nodes belongs to the same genotype found in the correspondent brain, but show several differential point mutations including M gene higher degree of biased U to C hypermutation. From these data and our observation that primary MV isolates in PBMC from acute measles may persist in, human lymphoblastoid cells we would discuss a possible mechanism for initiation of MV persistence infection .

220 **Fatal encephalitis in African green monkeys and common marmosets after aerosol infection with Rift Valley Fever virus.**

Douglas Reed, Diana Powell, Laura Bethel, Amy Caroline, Anita Trichel, Timothy Oury, and Amy Hartman *University of Pittsburgh*

Rift Valley Fever virus (RVFV) outbreaks occur in eastern Africa and the Middle East, with epidemics of severe disease in livestock and human populations. In humans, RVFV causes a self-limiting febrile illness, but a small percentage of people develop severe complications including hepatitis, retinitis, hemorrhagic fever, and encephalitis. RVFV is infectious by many routes including inhalation. In an effort to develop a nonhuman primate model of aerosol exposure to RVFV, African green monkeys (AGM) and common marmosets were exposed to small particle aerosols containing RVFV. In both species, a biphasic fever response was seen which corresponded with a decline in activity levels. Three of four AGM and four of eight marmosets developed neurological signs (drooling, unsteady gait, seizures), became moribund and were promptly euthanized. Gross pathological findings of both species included damage to the liver and kidneys and vasculitis in the brain; evidence of hemorrhage was found in the lungs of 2 AGM. AGMs and marmosets both had elevations in alkaline phosphatase, blood urea nitrogen, and white blood cells at time of necropsy. The elevation in white blood cell counts was predominantly granulocytes. Glucose levels were elevated in the three AGM and two of the three had thrombocytopenia. Virological and pathological findings confirmed viral encephalitis in both AGMs and marmosets. Both AGM and marmosets appear to be good models of the encephalitis caused by RVFV. Additional clinical findings in AGM (thrombocytopenia, hemorrhaging in the lung) suggest that AGM might be a good model for exploring other aspects of RVFV disease.

221 **Avian Influenza Virus Hemagglutinins H2, H4, H8, and H14 support a highly pathogenic phenotype**

Olga Stech, Jutta Veits, El-Sayed Abdelwhab, Ute Wessels, Siegfried Weber, Angele Breithaupt, Marcus Gräber, Sandra Gohrbandt, Jessica Bogs, Jens P. Teifke, Thomas C. Mettenleiter, Juergen Stech
Friedrich-Loeffler-Institut

Highly pathogenic avian influenza viruses (HPAIV) evolve from low-pathogenic precursors with hemagglutinin (HA) serotypes H5 or H7 by acquisition of a polybasic HA cleavage site (HACS). Since the reason of this serotype restriction is still unclear, we aimed to distinguish between compatibility of a polybasic HACS with H5/H7 HA only and unique predisposition of H5/H7 for insertion mutations. Engineered polybasic HACS mutants from several low-pathogenic avian strains (LPAIV) of serotypes H5N1, H3N8, H9N2, and H4N6 did not exhibit high virulence in chicken. Therefore, we generated HA reassortants by introducing a polybasic HACS into the HA of several LPAIV with serotypes H1, H2, H3, H4, H6, H8, H10, H11, H14 or H15, and co-transfection with either H9N2 LPAIV or H5N1 HPAIV genes. Reassortants containing the engineered H2, H4, H8 or H14 in the HPAIV background were lethal and exhibited intravenous pathogenicity indices of 2.79, 2.37, 2.85, and 2.61 respectively, equivalent to natural HPAIV. Thus, in case of a polybasic HACS, nonH5/H7 HA can enable high virulence in the HPAIV background. Then, we further mapped those HPAIV virulence determinants by generating several reassortants from a H5N1 LPAIV and a HPAIV strain and found that the HPAIV HA and NA alone enable 100% lethality and efficient transmission to contact chickens. Thus, beside an HA with polybasic HACS, the NA is a second essential virulence determinant of H5N1 HPAIV in chicken. Overall, the restriction of natural HPAIV to H5 and H7 is likely due to a unique predisposition for acquisition of a polybasic HACS.

222 **Establishment and characterization of a highly efficient reverse genetics system for Henipavirus rescue**

Tatyana Yun (1), Arnold Park (2), Olivier Pernet (2), Terry L. Juelich (1), Jennifer Smith *(1), Lihong Zhang (1), Benhur Lee (2), Alexander N. Freiberg (1)*
1. Department of Pathology, University of Texas Medical Branch, Galveston, USA
2. Departments of Microbiology, Immunology & Molecular Genetics, University of California Los Angeles, Los Angeles, USA

We established a highly efficient reverse genetic system that enabled rescue of recombinant henipaviruses (rNiV/rHeV) entirely from cloned cDNAs without the need for vaccinia-driven T7-RNA polymerase (RNAP) or the use of BSR-T7 cells. Our rescue system uses codon-optimized T7-RNAP co-transfected with modified full-length cDNA clones and supporting plasmids encoding the nucleoprotein, phosphoprotein and polymerase genes. Engineering a self-cleaving hammerhead ribozyme between the T7 promoter and the 3' leader of the rNiV or rHeV genome increased rescue efficiency ≥3 logs: (1) 3'Rbz-mediated autocatalytic cleavage resulted in the exact 3' termini required for efficient antigenome-genome transcription/replication, and (2) allowed the inclusion of the transcript-initiating 3Gs in the full T7 promoter that is required for high efficiency T7-RNAP-mediated transcription. High efficiency rescue allowed us to rescue 15 rNiV/HeV expressing various fluorescent (eGFP/mCherry) and/or bioluminescent (firefly/Gaussia luciferase [f-luc/G-luc]) reporter genes, including dual-combination (fluorescent and bioluminescent) reporters. Many reporter rNiVs (or rHeV) reflected the replication and tropism of parental henipaviruses in primary endothelial and neuronal cells, and exhibited similar pathogenicity in the hamster model. rNiV-[f-luc] and rNiV-[G-luc] allowed for live animal imaging of NiV under BSL-4 conditions to monitor the progression of viral replication; determination of G-luc activity in serum enabled quantitative determination of rNiV-[G-luc] replication without the use of qPCR. Finally, HeV-F, HeV-G, or HeV-M, singly, or in all possible combinations, were able to complement their NiV counterparts and allowed for efficient rescue of rNiV-[mCherry]. Our system represents a powerful tool for more refined and sophisticated analyses of henipavirus pathogenicity and evolution.

223 **Characterization of virulence mechanisms of hemorrhagic fever-causing arenaviruses**

Yuying Liang, Hinh Ly *University of Minnesota, Twin Cities*

Several pathogenic arenaviruses, including Lassa virus, cause hemorrhagic fever (HF) infections that can result in significant morbidity and mortality in humans with limited preventative and treatment modalities. A hallmark of severe HF is the high levels of viremia coupled with generalized immune suppression of the hosts, the mechanisms for which are unknown. Recent studies in our laboratory using viral reverse genetic system, cell-based and biochemical assays and a small animal model for Lassa fever have revealed several potential mechanisms that arenaviruses use to cause virulent infection in the hosts (McLay, et al., 2013, JVI, 2012, Antiviral Res; Jiang, et al., 2013, JBC; Kumar, et al., 2012, Virol; Wang, et al., 2012, JVI; Qi, et al., 2010, Nature; Liang, et al., 2009, Ann NY Acad Sci; Lan, et al., 2009, JVI; Lan, et al., 2008, Arch Virol.). Specifically, each of the four viral gene products (GPC glycoprotein, L polymerase, NP nucleoprotein, and Z matrix protein) play important roles in mediating efficient pathogenic arenaviral entry, enhanced viral genome replication, and inhibition of cellular apoptosis, translation, and interferon beta (IFNβ) production. I will present some published data as well as novel results from our recent studies that aim at elucidating the roles of each of these viral proteins in mediating HF disease pathogenesis. Novel insights learned from these studies can be exploited for the development of novel therapeutics and vaccines against deadly HF infections (McLay, et al., 2012, Antiviral Research, invited review).

224 **Immunopathogenesis of Henipavirus infection: role of Interferon type I signalling**

Kevin Dhondt, Cyrille Mathieu, Marie Chalons, and Branka Horvat *INSERM U758, Human Virology, F-69365 France; Ecole Normale Supérieure de Lyon, Lyon, France; University of Lyon 1; 21 Avenue Tony Garnier, 69365 Lyon Cedex 07, France*

Hendra virus (HeV) and Nipah virus (NiV) are closely related, recently-emerged paramyxoviruses, belonging to the Henipavirus genus and capable of causing considerable morbidity and mortality in a number of mammalian species, including humans. However, in contrast to many other species, mice are resistant to henipavirus infection, although they do express functional virus entry receptors. We have characterized a new animal model of henipaviurs infection in mice deleted in Interferon-Type I Receptor (IFNAR KO). We showed that in contrast to wild type (wt) mice, these mice are highly susceptible to NiV infection by different routes of infection and develop fatal encephalitis with pathology and immunohistochemical features similar to what was found in humans. Sublethaly infected mice developed virus-specific neutralizing antibodies. Surprisingly, the resistance to NiV was completely preserved in mice deleted for sensors of the innate immune system, including TLR3, suggesting the importance of constitutive, rather than virus-induced IFN-I signaling in the control of NiV infection. Furthermore, while infection seems to be stopped at very early stages in intraperitoneally injected wt mice, the intracranial NiV infection leads in these mice to systemic spread of the virus with lethal outcome. These data suggested that particular IFN-I producing cells may play the important role in the early control of NiV infection. Altogether, these results reveal IFNAR-KO mice as a new small animal model to study HeV and NiV pathogenesis, prophylaxis and treatment and suggest the critical role of IFN-I signaling in viral immunopathogenesis, opening thus new perspectives to control this highly lethal infection.

225 **Rabies virus-induced expression of chemokines/cytokines enhances the blood-brain-barrier (BBB) permeability in mice**

Qing Q. Chai1,2, Wen Q. He2, Zhen F. Fu1,2
1State-key Laboratory of Agricultural Microbiology, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, 430070, China
2Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA 30602

Enhancement of blood-brain-barrier (BBB) permeability has been found to be an important factor in clearing rabies virus (RABV) from the central nervous system (CNS). However, the mechanism by which RABV infection enhances the BBB permeability is not understood. In the present study, two RABV strains were used to study the mechanisms by which RABV infection results in the enhancement of BBB permeability. Mice were infected with either a lab-adapted or a wild-type RABV and the BBB permeability as well as the expression of tight junction proteins was measured in the mice after infection. It was found that only lab-adapted RABV induced the enhancement of BBB permeability in infected mice and it is accompanied by induction of inflammatory responses. The level of tight-junction proteins (ZO-1, Claudin-5, Occludin) was significantly reduced in the brain of mice infected only with lab-adapted RABV, suggesting that the enhancement of BBB permeability is associated with reduction in the expression of tight-junction proteins. To investigate what modulate the expression of tight-junction proteins, human and mouse Brain Microvascular Endothelial Cells (H/MBMECs) were infected with each of the viruses and it was found that RABV neither infected the brain endothelial cells nor modulated expression of the tight-junction proteins. However, brain extract prepared from mice infected with lab-adapted, but not with wild-type RABV reduced the expression of tight-junction proteins. Luminex assay revealed that the extract from lab-adapted RABV-infected brain has higher levels of cytokine/chemokines than that from wild-type RABV-infected brain. These studies thus indicate that the enhancement of BBB permeability and the reductions of tight junction proteins are not due to RABV infection per se but most likely due to the virus-induced inflammatory cytokines/chemokines.

226 **A 3D organotypic lung tissue model to study hantavirus pulmonary syndrome**

Karin B. Sundström1,2, Anh Thu Nguyen Hoang3, Puran Cheng3 Shawon Gupta1,2, Clas Ahlm4, Mattias Svensson3 and Jonas Klingström1,2,3
1 Department of Microbiology, Tumor and Cell Biology, Karolinska Insitutet, SE-171 77 Stockholm, Sweden
2 Department of Preparedness, Swedish Institute for Communicable Diseases Control, SE-171 82 Solna, Sweden
3 Center for Infectious Medicine, Department of Medicine, Karolinska Insitutet, Karolinska University Hospital Huddinge, SE-141 86 Stockholm, Sweden
4 Department of Clinical Microbiology, Division of Infectious Diseases, Umeå University, SE-901 85 Umeå, Sweden

Andes virus (ANDV) is a rodent-borne hantavirus that causes hantavirus pulmonary syndrome (HPS) with case fatality rate around 40%. It is not well known why hantaviruses cause disease in humans. In particular, very little is known regarding the effect hantaviruses may have at the initial site of infection during the incubation period, and why infection change from asymptomatic to a life-threatening disease in humans. Here, we used a 3-dimensional organotypic model of human lung tissue to investigate early and long-term effects of ANDV-infection. Sudden increase in progeny virus production was observed approximately 10-15 days after infection, coinciding with induction of a short lived-type I and type III interferon response. Furthermore, the peak in viral production was followed by increased levels of extracellular IP-10, IL-6, IL-8 and VEGF-A, and decreased levels of RANTES in infected models. Caspase 3 was not activated by infection, suggesting that ANDV does not induce apoptosis in this model. Late after infection lower levels of extracellular cytokeratin 18, a marker for epithelial cell death, were observed in infected models indicating that ANDV might impact epithelial cell survival. This indicates that direct ANDV-induced pro-inflammatory cytokine responses and VEGF-A production in the lungs might be involved in HPS-pathogenesis. We also observed that addition of dentritic cells in the model had an antiviral effect on ANDV-infection.

227 **The incubation period is longer than the latent period in chickens and turkeys experimentally infected with Highly Pathogenic Avian influenza viruses**

James Seekings, Chad Fuller, Caroline Warren, June Mynn, Ross Cooper, Elizabeth Aldous, Richard M. *Irvine and Ian H. Brown AHVLA –*

In the European Union, and many other countries world-wide, control measures for highly pathogenic avian influenza (HPAI) are imposed by legislation and involve stamping out policies with defined restriction zones and severe trade implications. Early detection of outbreaks can minimise losses and cost through reducing potential spread. This work generated experimental data of first clinical signs in chickens and turkey’s infected with HPAI and the relationship between the incubation period and latent period. Ten 3-week-old chickens and turkeys were infected with either H7N1 or H5N1 HPAI, using a dose 0.5 log10 above the previously determined MLD50, via the intraocular and intra nasal route. All of the birds were inspected regularly and clinical signs observed and scored. Buccal and cloacal swabs were taken up to three times a day and tested using real time RT-PCR. Indices were calculated for clinical signs as well as buccal and cloacal virus shedding to allow comparison of the latency and incubation periods. For both H7 and H5 in chickens and turkeys the latent period was shorted than the incubation period indicating a period of subclinical infection of approximately 12 hours. The high risk period between infection and the onset of observed clinical signs was less than 28 hours with 100% mortality by 48 hours. </p>

228 **Elements in the Canine Distemper Virus M 3' UTR Contribute to Control of Replication Efficiency and Virulence**

Danielle E. Anderson, Alexandre Castan, Martin Bisaillon, Veronika von Messling

1 Emerging Infectious Diseases Program, Duke-NUS Graduate Medical School, Singapore, Singapore.

2 INRS-Institut Armand-Frappier, University of Quebec, Laval, Quebec, Canada.

3 University of Sherbrooke, Sherbrooke, Quebec, Canada.

Canine distemper virus (CDV) is a negative-sense, single-stranded RNA virus within the genus *Morbillivirus* and the family *Paramyxoviridae*. The *Morbillivirus* genome is composed of six transcriptional units that are separated by untranslated regions (UTRs), which are relatively uniform in length, with the exception of the UTR between the matrix (M) and fusion (F) genes. This UTR is at least three times longer and in the case of CDV also highly variable. Exchange of the M-F region between different CDV strains did not affect virulence or disease phenotype, demonstrating that this region is functionally interchangeable. Deletions in the M 3' UTR resulted in gradual reduction of virulence, while replacement of the deletion with a random sequence led to complete attenuation, suggesting that overall length as well as partially redundant sequence motifs distributed throughout the region contribute to virulence.

229 **Pathogenicity of H5N1 highly pathogenic avian influenza virus in wild ducks and Herons**

Kosuke Soda1,2, Tatsufumi Usui1,2, Yukiko Uno1, Yasuko Nagai1, Hiroichi Ozaki1,2, Hiroshi Ito1,2, Naoki Yamamoto3, Tomokazu Tamura3, Takahiro Hiono3, Masatoshi Okamatsu3, Yoshihiro Sakoda3, Ayato Takada4, Tsuyoshi Yamaguchi1,2, Toshihiro Ito1,2
1 Department of Veterinary Medicine, Faculty of Agriculture, Tottori University, Tottori, Japan
2 Avian Zoonoses Research Center, Faculty of Agriculture, Tottori University, Tottori, Japan
3 Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido, Japan
4 Research Center for Zoonosis Control, Hokkaido University, Sapporo, Hokkaido, Japan

Widespread outbreaks of highly pathogenic avian influenza (HPAI) caused by H5N1 viruses occurred in wild birds in Japan from 2010-11. To estimate the risk of wild birds as a source of virus infection in the environment, we examined the pathogenicity of a causal H5N1 HPAI virus to *Anatidae* (mallards, wigeons, pintails, tufted ducks and mandarin ducks) and *Ardeidae* (grey herons, intermediate egrets, little egrets and black-crowned night herons). A tufted duck (1/2 : indicates 1 out of 2 birds), a mandarin duck (1/3), grey herons (2/4), little egrets (2/2) and a black-crowned night heron (1/3) showed clinical signs such as depression and neurologic symptoms after intranasal inoculation with 106 EID50 of A/mandarin duck/Miyazaki/22M807-1/2011 (H5N1), which belonged to clade 2.3.2.1 and are mainly circulating in East Asia. All the birds presenting disease signs terminated in death except one tufted duck and one grey heron. Viruses were mainly recovered from the laryngopharyngeal swabs of mallards, tufted ducks, mandarin ducks and *Ardeidae* until 5-7 days post inoculation. On the other hand, viruses were rarely recovered from the swabs of wigeons and pintails. Further study revealed that the H5N1 HPAI viruses were transmitted between *Anatidae* or grey heron populations. Together, these results showed that pathogenicity of the H5N1 HPAI viruses differed by bird species. The viruses were lethal to some birds, indicating that they have the potential to disseminate the virus to other bird species and environment. Therefore, wild birds should be kept out of poultry farms to prevent HPAI outbreaks in the future.

230 **Interferon type I and T cells determine susceptibility of mice to various Old World Arenaviruses**

Toni Rieger 1, Doron Merkler 2, Lisa Oestereich 1, and Stephan Günther 1
1 Department of Virology, Bernhard-Nocht-Institute for Tropical Medicine, Bernhard-Nocht-Str. 74, 20359 Hamburg, Germany
2 Geneva University Hospital Department of Pathology and Immunology, Geneva Faculty of Medicine Centre Médical Universitaire 1, rue Michel Servet 1211 Geneva, Switzerland

Several members of the Old World arenaviruses are endemic in Sub-Saharan Africa. Lassa virus, Mopeia virus, and Morogoro virus share the same rodent host (*Mastomys natalensis*), while Mobala virus is carried by *Praomys* rodents. However, only Lassa virus (LASV) is pathogenic to humans; it causes Lassa fever in humans, a haemorrhagic disease endemic in West Africa. The related Mopeia, Morogoro, and Mobala viruses are not associated with disease in humans. As non-transgenic mice are not susceptible to these African arenaviruses, we tested whether mice with deficiency in the type I interferon receptor (IFNAR-KO) can be productively infected and may serve as an animal model for these viruses. Various strains of Lassa virus replicated to high titres in IFNAR-KO mice, while Mopeia, Morogoro, and Mobala virus replicated hardly or not at all. Lassa virus-infected IFNAR-KO mice also showed biochemical and histopathological signs of disease. Thus, the virulence of the viruses in humans is reflected in IFNAR-KO mice, which may serve as a useful model to study viral determinants of Lassa fever. The data also demonstrate that the main hurdle to productive infection of mice by Lassa virus is the interferon response. Additional depletion of CD4+ and CD8+ T cell in IFNAR-KO mice facilitated high-level replication also of the apathogenic viruses, indicating that the T cell response plays a major role in host restriction to these viruses.

231 **Ebola virus targets Nrf2-dependent antioxidative stress response via its structural protein VP24.**

St Patrick Reid, Kirill Nemirov, Mathieu Mateo, Audrey Page and Viktor Volchkov.
Molecular basis of high pathogenicity of viral infections, INSERM CIRI U1111, Lyon France

Ebola virus (EBOV) causes severe hemorrhagic fever in human and non-human primates and is characterized by dysregulated inflammatory responses. In this study we demonstrate that replication of EBOV is associated with the inhibition of Nrf2 (Nuclear factor erythroid-related factor-2)-dependent cellular pathway. This pathway determines maintenance of intracellular redox balance, playing an important role in cell protection against different environmental insults and is also involved in regulation of cellular response to inflammation. The data obtained indicate that structural protein VP24 is responsible for inhibition of the Nrf2 function and this activity is independent of its interferon signalling antagonism. We speculate that novel VP24 function contributes to high pathogenicity of EBOV infection.

232 **Length of the NS1 linker region determines pathogenicity of a highly pathogenic H5N1 influenza A virus**

Mirco Schmolke1, Benjamin G Hale1, 2, Rong Hai1, Jianqiang Ye3, Balaji Manicassamy1, Daniel R Perez3, Adolfo García Sastre1
1 Icahn School of Medicine at Mount Sinai, Department of Microbiology, New York, NY, USA
2 University of Glasgow, Institute of Infection, Immunity and Inflammation, Glasgow, UK
3 University of Maryland, Department of Veterinary Medicine, College Park, MD, USA

The non structural protein 1 (NS1) of influenza A viruses (IAV) is a major viral pathogenicity factor. It is a multifunctional protein that blocks host cell mRNA maturation, splicing, activates PI3K signaling and interferes with RIG-I dependent recognition of viral RNA by the cytosolic RNA sensor RIG-I. NS1 is composed of a N-terminal RNA-binding domain (RBD), a central effector domain (ED) and a C-terminal unstructured tail domain. RBD and ED are connected by a flexible linker region, which presumably allows the two domains to arrange in different 3D conformations, depending on the cellular context and cellular or viral encoded interacting proteins/RNA. Interestingly, in some H5N1 influenza A NS1s from human cases this linker region is lacking 5aa. We were curious to see if this deletion is a species specific adaptation and tested the viral replication and pathogenicity in mammalian and avian host model systems.We show that reintroduction of these deleted amino acids can indeed impact pathogenicity of influenza A/Viet Nam/1203.

233 **Substitutions T200A and E227A in the hemagglutinin of pandemic 2009 influenza A virus increase lethality but decrease transmissi**

Carles Martínez-Romero1)3), Erik de Vries4), Alan Belicha-Villanueva1)3), Ignacio Mena1)3), Donna M. Tscherne1)3), Virginia L. Gillespie4), Randy A. Albrecht1)3), Cornelis A. M. de Haan5), Adolfo García-Sastre1)2,)3)

1) Department of Microbiology, 2) Department of Medicine, Division of Infectious Diseases, 3) Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA, 4) Center for Comparative Medicine and Surgery, Comparative Pathology Laboratory, Icahn School of Medicine at Mount Sinai, New York, NY, USA, 5) Virology Division, Department of Infectious Diseases & Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

We describe that swine influenza virus-like substitutions T200A and E227A in the hemagglutinin of the 2009 pandemic influenza virus alter its pathogenesis and transmission. Viral replication is increased in mammalian cells. Infected mice show increased disease as measured by weight loss and lethality. Transmission in ferrets is decreased in the presence of both substitutions, suggesting that 200T and 227E HA amino acids are adaptive changes in the HA of swine-origin influenza viruses associated with increased transmission and decreased pathogenesis.

234 **Pathogenicity of a pH1N1 influenza virus isolated from a fatal case. Role of host genetics in the outcome of the infection**

Ana Falcon 1 2, Ariel Rodríguez 1 2, Maite Cuevas 3, Francisco Pozo 3, Inmaculada Casas 3, Juan Ortín 1 2, Amelia Nieto 1 2

1: National Center of Biotechnology (CNB-CSIC) Madrid, Spain
2: CIBER Enfermedades Respiratorias, Mallorca, Spain
3: National Center of Microbiology, ISCIII, Madrid, Spain

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 co-morbid 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. Genetic characterization of M and F viruses showed difference in 29 nucleotides distributed over all the genome, 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. With regard to knowledge of the host characteristics, a deleted form of chemokine receptor 5 (CCR5), CCR5Δ32, affect the patient´s response to the infections. Thereby, examination of CCR5 genotype of M and F virus-infected patients was performed. F virus-infected patient was homozygous for CCR5Δ32. Additionally, a population study revealed an increased presence of CCR5Δ32 allele in fatal cases among Spanish patients with confirmed diagnosis of infection with A(H1N1)pdm09 virus.

235 **Influenza HA subtypes demonstrate divergent phenotypes for cleavage activation and pH of fusion: implications for host range and**

Summer E. Galloway1, Mark L. Reed2, Charles J. Russell2,3, David A. Steinhauer1
1Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, Georgia, USA; 2Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, Tennessee, USA; 3Department of Microbiology, Immunology, and Biochemistry, University of Tennessee Health Science Center, Memphis, Tennessee, USA

The IAV HA protein must be activated by host cells proteases in order to prime the molecule for fusion. Consequently, the availability of activating proteases and the susceptibility of HA to protease activity represents key factors in facilitating virus infection. As such, understanding the intricacies of HA cleavage by various proteases is necessary to derive insights into the emergence of pandemic viruses. To examine these properties, we generated a panel of HAs representative of the 16 subtypes that circulate in aquatic birds, as well as those that have infected humans. We examined the susceptibility of the panel of HA proteins various proteases, as well as the pH at which these HAs mediated membrane fusion, as this property is related to the stability of the HA molecule and influences the capacity of influenza viruses to remain infectious in natural environments. Our results show that cleavage efficiency of HA can vary significantly and some HA subtypes display stringent selectivity for specific proteases. Additionally, we found that the pH of fusion varies by 0.7 pH units among the subtypes, and notably, we observed that the pH of fusion for HAs from human isolates was as much as 0.5 pH units lower than that observed from avian isolates of the same subtype. Overall, these data provide the first broad-spectrum analysis of cleavage-activation and membrane fusion characteristics for all of the IAV HA subtypes, and also show that there are substantial differences between the subtypes that may influence transmission and establishment in new species.

236 **Modeling the Nipah virus transmission cycle**

Emmie de Wit, Joseph B. Prescott, Darryl Falzarano, Trenton Bushmaker, Dana Scott, Vincent J. Munster, Heinz Feldmann *Laboratory of Virology, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, USA*

Since 2001, outbreaks of Nipah virus (NiV) have occurred almost yearly in Bangladesh with case-fatality rates up to 90%. Epidemiological data suggest that in Bangladesh, NiV is transmitted from fruit bats to humans via consumption of raw date palm sap contaminated by bats during collection, with subsequent human-to-human transmission. To model this transmission cycle of NiV, we determined the viability of NiV in artificial date palm sap. Virus titers remained stable for >3 days, thus potentially allowing food-borne transmission. Next, we modeled food-borne NiV infection by supplying Syrian hamsters with artificial date palm sap containing NiV. Hamsters were monitored for signs of disease; sequential necropsies were performed to analyze virus distribution in 17 tissues and swabs were collected daily to analyze virus shedding. Depending on the dose, drinking of NiV resulted in neurological disease in up to 63% of hamsters, indicating that food-borne infection with NiV can indeed occur. Virus shedding was observed in all hamsters on several days after drinking. To model human-to-human transmission, we first determined that transmission of NiV occurs through direct contact in 25% of hamsters pon intranasal inoculation. Contact transmission of NiV upon drinking of contaminated date palm sap occurred in 8% of animals. Understanding the NiV transmission cycle is essential for mitigating NiV outbreaks. The limited potential for medical intervention in resource-poor outbreak areas highlights the need for preemptive strategies, like educational outreach, focused at preventing transmission. This research was supported by the Intramural Research Program of the NIH, NIAID.

237

PATHOGENICITY FUNCTIONS OF LYSSAVIRUS GLYCOPROTEINS

Tobias Nolden, Jens Peter Teifke, Stefan Finke
Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Greifswald – Riems

Of the twelve Lyssavirus species, only rabies virus (RABV) circulates in carnivore populations. Other lyssaviruses are restricted to bat hosts. Rabies disease in non-chiropteran mammalians caused by bat-associated lyssaviruses is occasionally observed. However, such spill-over infections are considered as dead-end infections without further spread in the non-bat host population. Although host restriction of lyssaviruses is obvious, the molecular mechanisms involved are unknown. As the viral glycoprotein G is a major pathogenicity determinant and contributes to host tropism, we investigated the impact of different lyssavirus G proteins on virus replication, virulence and pathogenicity. Chimeric viruses were generated in which the ectodomain of G proteins from several RABV isolates and bat-associated lyssaviruses were inserted into an attenuated RABV genetic background. Most of the chimeras grew to reasonable titers in cell culture and intracranial infections of Balb/c mice verified *in vivo* replication and disease induction. Notably, in contrast to the original, attenuated RABV, only a portion of the chimeras expressing G sequences from virulent RABV or bat virus isolates exhibited increased pathogenicity after peripheral inoculation of the gluteal muscle. Thus, replacement of the G ectodomain can be sufficient to increase the pathogenic potential of an attenuated RABV in mice, but not necessarily leads to an increased virulence, even when adopted from virulent isolates. The identification of "virulent" and "non-virulent" G sequences now allows the targeted dissection of G sequences crucial for neuroinvasion, pathogenicity and host adaptation in the mouse model.

238

Analysis of the Immune Response to Sin Nombre Hantavirus in the Rhesus Macaque Model of Hantavirus Cardiopulmonary Syndrome

Joseph Prescott, David Safronetz, Heinz Feldmann
Laboratory of Virology, DIR, NIAID, NIH, Rocky Mountain Laboratories, Hamilton, MT, 59840

Pathogenic New World hantaviruses cause a disease termed hantavirus cardiopulmonary syndrome (HCPS). The primary agent of HCPS in North America is Sin Nombre virus. Humans that succumb to infection have high amounts of pro-inflammatory cytokines in the lungs, and although hantaviruses primarily infect endothelial cells, infection is non-cytopathic. Therefore, it is thought that HCPS has an immunopathogenic component. In an effort to develop animal models for HCPS caused by SNV, we inoculated Rhesus macaques and monitored signs of disease. Six of 8 animals developed severe disease strikingly similar to HCPS and were euthanized 16-22 days post-inoculation. Peripheral blood mononuclear cells (PBMC) were isolated throughout the course of infection for flow cytometric analysis. CD8+ T-cells increased dramatically during late stages of disease. These cells showed an effector memory phenotype. There was also a large increase in the proportion of CD8+ T-cells expressing proliferation and activation markers, and in cell percentages expressing granzyme B. Neutrophilia is often observed in HCPS. Analysis of whole blood and PBMCs showed an increase in neutrophils and granulocytes only in macaques that developed disease. Lymphocytes from infected animals that were stimulated with antigen ex vivo showed robust production of cytokines. Macaques that developed disease expressed high levels of cytokines in the lungs immunohistochemically, primarily originating from alveolar macrophages. The kinetics of immune activation suggests that a robust response is elicited, coinciding with disease onset, and this response might contribute to disease via an immunopathogenic mechanism. This model provides an insight into the immunological events leading to HCPS.

239 **Role of Neutrophils in the Induction of Pulmonary Edema during Hantavirus Infection in C.B-17Scid Mice**

Jiro Arikawa1, Takaaki Koma1, Kumiko Yoshimatsu1, Noriyo Nagata2, Yuko Sato2, Kenta Shimizu1, Takako Amada1, Sanae Nishio1, and Hideki Hasegawa2
1 Department of Microbiology, Graduate School of Medicine, Hokkaido University, Kita-ku, Kita-15, Nishi-7, Sapporo 060-8638, Japan
2 Department of Pathology, National Institute of Infectious Diseases, 4-7-1 Gakuen Musashi-Murayama, Tokyo 208-0011, Japan

Increased vascular permeability, pulmonary edema and neutrophilia are considered as common phenomenon relate to pathogenesis of hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). Here, we examined the role of neutrophils in the induction of pulmonary edema in hantavirus infection by using mouse model.C.B-17/Icr-scid/scidJcl (SCID) mice (8-week-old female Nihon Clea, Tokyo, Japan) were inoculated with Hantaan virus cl-1 (HTNV, 4000 FFU) by intraperitoneal injection. The degree of pulmonary edema was assessed by calculation of the ratio of alveoli with exudate. Neutrophil depletion was performed by administration of the anti-Gr-1 antibody. Pulmonary vascular permeability was analyzed by the Evans blue extravasation method.Progressive weight loss, slowing of activity and ruffled fur were appeared after 21 days post-inoculation (dpi). Marked histopathological change was observed in the lung. Cellular proliferation was observed in the lung. Adhesion of neutrophils, degenerated and multilayered endothelial cells were seen in the middle size vein. Pulmonary edema began to be observed from 28 dpi and peaked at 35 dpi then decreased in accorded with the increased pulmonary permeability detected by Evans blue extravasation. Increases in neutrophils in the lung and blood were observed when pulmonary edema began to be observed. The occurrence of pulmonary edema was inhibited by neutrophil depletion. Moreover, the pulmonary vascular permeability was also significantly suppressed by neutrophil depletion in the infected mice. Thus, these results indicate that neutrophils play an important role in the appearance of pulmonary vascular hyperpermeability and the occurrence of pulmonary edema after hantavirus infection in SCID mice.

240 **Antigenic and chicken embryonic phenotype differences between Korean-like and G1 lineage H9N2 avian influenza viruses**

Bethany Nash, Alejandro Núñez, Daniel Hicks, Sharon M. Brookes, Ian H. Brown
Animal Health and Veterinary Laboratories Agency, Woodham Lane, New Haw, Surrey, KT15 3NB

H9N2 is endemic in poultry in mainland China and Hong Kong with evidence that pigs and humans are susceptible to infection suggesting pandemic potential. Antigenic characterisation of H9N2 viruses circulating in avian populations demonstrated clustering consistent with geograhic distribution and phylogeny with evidence of low cross-protection between clusters as demonstrated by cross-Heamagglutination Inhibition (HI). The Korean-like lineage, shows no cross reaction with the G1 or Beijing clusters. We investigated H9N2 virus distribution in tropism of H9N2s within chicken embryos as a model of pathogenicity for poultry. A/quail/Hong Kong/G1/1997 (quHK97), G1 lineage reference strain or A/mallard/England/7798-6499/2006 (malEng06), Korean lineage, were innoculated into 14-day-old embryonic chicken eggs via the allantoic route at 102 or 105 EID50. Duplicate whole embryos were fixed in buffered formalin at 24, 48 and 72 hpi. Virus localisation was determined by immunohistochemistry against influenza A nucleoprotein. Both viruses showed similar distribution at 24 and 48 hpi with virus restricted to the allontoic epithelium. However, at 72hpi quHK97 also had consistent infection of the embryonic respiratory and digestive epithelium and skin, whereas malEng06 tropism was limited to a small number of allantoic epithelial cells in most embryos. These data suggest that malEng06, representing the Korean-like lineage, is antigenically and phenotypically distinct from the G1 and Beijing lineage viruses, which has implications for pandemic-preparedness vaccine strain selection.

241 **Genetic Determinants of Junin Virus Attenuation**

Alexey V. Seregin, Nadezhda E. Yun, Slobodan Paessler
Galveston National Laboratory, Department of Pathology, University of Texas Medical Branch, Galveston, TX

The New World arenavirus Junin (JUNV) is the causative agent of Argentine hemorrhagic fever (AHF) that is associated with high morbidity and significant mortality. A highly attenuated vaccine strain (Candid #1) was generated and used to vaccinate human population at risk. The identification of genetic determinants associated with Candid #1 attenuation would contribute to the development of better vaccines and therapeutics. We used a reverse genetics approach to rescue from cloned cDNAs the pathogenic Romero (rRomero) and the attenuated Candid #1 (rCandid) strains of JUNV. Using our cDNA-based reverse genetics system, we generated a set of rJUNVs: intersegment chimeric viruses rRomL/CamS and rCanL/RomS containing one genomic RNA segment originated from Romero and the other from Candid #1, rRomero viruses rRom/CanGPC and rRom/CanNP where ORF for either GPC or NP gene, respectively, was substituted with ORF for the corresponding gene of Candid #1, and rRom/G2/F427I virus that contains F427I substitution in the transmembrane region of G2 glycoprotein that has recently been demonstrated to significantly attenuate JUNV in a mouse model after intracranial inoculation. rRomL/CanS virus produced small plaques on Vero cells similar in size and morphological appearance to rCandid virus plaques. In contrast, the large plaques produced by rCanL/RomS and rRom/G2/F427I viruses were similar to the ones produced by rRomero. rRom/CanGPC and rRom/CanNP produced plaques of medium size. In one-step growth assays carried out at an MOI of 5 all chimeric viruses exhibited similar growth properties in Vero and A549 cells to the parental rRomero and rCandid viruses.

242 **Role of different regions of Newcastle disease virus fusion protein for its Pathogenicity**

Sandra Warlich(1), Christian Grund(2), Denis Kühnel, Thomas C. Mettenleiter(1), Angela Römer-Oberdörfer(1)
(1)Institute of Molecular Biology,(2)Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Südufer 10, D-17493 Greifswald-Insel Riems, Germany

Newcastle disease virus (NDV), the causative agent of a notifiable disease of poultry, exhibits different levels of pathogenicity, dependent on the virus strain. However, the molecular determinants of NDV virulence are not fully understood. The efficiency of proteolytic cleavage of the fusion protein (F) which is determined by presence or absence of a polybasic cleavage site, has long been considered a major determinant of NDV virulence. However, especially pigeon type paramyxovirus-1 (PPMV-1) isolates can exhibit low pathogenicity despite presence of a polybasic F cleavage site. Substitution of the genes encoding surface glycoproteins F and hemagglutinin-neuraminidase (HN) of a lentogenic (low virulence) NDV Clone 30 by those of a mesogenic (intermediate virulence) PPMV-1 (isolate R75/98) resulted in a recombinant NDV which possesses a polybasic F cleavage site (112RRKKR*F117), but low pathogenicity, demonstrated by an intracerebral pathogenicity index (ICPI) of 0.1. Substitution of only the Clone 30 F gene by that of PPMV-1 resulted also in a lentogenic recombinant NDV with an ICPI of 0.6, whereas the substitution of only the NDV Clone 30 sequence motif at the F cleavage site 112GRQGR*L117 by that of PPMV-1 R75/98 112RRKKR*F117 resulted in a recombinant NDV with an ICPI of 1.36, indicating a mesogenic virus. The stepwise substitution of selected sequence regions of the F gene of NDV Clone 30 by those of PPMV-1 R75/98 and the characterization of the respective recombinant viruses demonstrated that the cytoplasmic tail of the F protein plays an important role in NDV pathogenicity in this context.

243 **Molecular diagnostics for Lassa fever at Irrua Specialist Teaching Hospital, Nigeria**

Danny A. Asogun 1, Donatus I. Adomeh 1, Jacqueline Ehimuan 1, Ikponmwonsa Odia 1, Meike Hass 2, Martin Gabriel 2, Stephan Olschlager 2, Beate Becker-Ziaja 2, Onikepe Folarin 3, Eric Phelan 4,5, Philomena E. Ehiane 1, Veritas E. Ifeh 1, Eghosasere A. Uyigue 1, Yemisi T. Oladapo 1, Ekene B. Muoebonam 1, Osagie Osunde 1, Andrew Dongo 6, Peter O. Okokhere 7, Sylvanus A. Okogbenin 8, Mojeed Momoh 8, Sylvester O. Alikah 9, Odigie C. Akhuemokhan 9, Peter Imomeh 10, Maxy A. C. Odike 11, Stephen Gire 4,5, Kristian Andersen 4,5, Pardis C. Sabeti 4,5, Christian T. Happi 3, George O. Akpede 9, Stephan Gunther 2

1 Institute of Lassa Fever Research and Control, Irrua Specialist Teaching Hospital, Edo State, Nigeria, 2 Department of Virology, Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Germany, 3 Malaria Research Laboratories, College of Medicine, University of Ibadan, Ibadan, Nigeria, 4 Broad Institute, Cambridge, Massachusetts, United States of America, 5 Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts, United States of America, 6 Accident and Emergency Unit, Irrua Specialist Teaching Hospital, Edo State, Nigeria, 7 Department of Medicine, Irrua Specialist Teaching Hospital, Edo State, Nigeria, 8 Department of Obstetrics and Gynecology, Irrua Specialist Teaching Hospital, Edo State, Nigeria, 9 Department of Pediatrics, Irrua Specialist Teaching Hospital, Edo State, Nigeria, 10 Department of Family Medicine, Irrua Specialist Teaching Hospital, Edo State, Nigeria, 11 Department of Clinical Pathology, Irrua Specialist Teaching Hospital, Edo State, Nigeria

Lassa fever is a viral hemorrhagic fever endemic in West Africa. However, none of the hospitals in the endemic areas of Nigeria has the capacity to perform Lassa virus diagnostics. Case identification solely relies on non-specific clinical criteria. The Irrua Specialist Teaching Hospital (ISTH) struggled with this challenge for many years. A laboratory for molecular diagnosis of Lassa fever, complying with basic standards of diagnostic PCR facilities, was established at ISTH in 2008. During 2009 through 2010, samples of 1,650 suspected cases were processed; 198 (12%) tested positive by Lassa virus RT-PCR. No remarkable demographic differences were observed between PCR-positive and negative patients. The case fatality rate for Lassa fever was 31%. Nearly two thirds of confirmed cases attended the emergency departments of ISTH. The time window for therapeutic intervention was extremely short, as 50% of the fatal cases died within 2 days of hospitalization--often before ribavirin treatment could be commenced. Fatal Lassa fever cases were older ($p = 0.005$), had lower body temperature ($p<0.0001$), and had higher creatinine ($p<0.0001$) and blood urea levels ($p<0.0001$) than survivors. Lassa fever incidence in the hospital followed a seasonal pattern with a peak between November and March. Lassa fever case management was improved at a tertiary health institution in Nigeria through establishment of a laboratory for routine diagnostics of Lassa virus. Data collected in two years of operation demonstrate that Lassa fever is a serious public health problem in Edo State and reveal new insights into the disease in hospitalized patients.

244 **Rabies virus phosphoprotein gene functions to facilitate viral neuroinvasion by viral replication in muscle.**

Satoko Yamaoka1), Naoto Ito1)2), Keisuke Nakagawa1), Kazuma Okada1), Kota Okadera1), Makoto Sugiyama1)2)
1)The United Graduate School of Veterinary Sciences, Gifu University
2)Laboratory of Zoonotic Diseases, Faculty of Applied Biological Sciences, Gifu University

Rabies virus (RABV) is a highly neurotropic pathogen that causes severe neurological symptoms and death in humans and animals. RABV is usually transmitted via a bite wound caused by a rabid animal and then infects peripheral nerves before spreading to the central nervous system. Despite the importance of the infection process at a peripheral site in the pathogenesis of rabies, little is known about the mechanism of peripheral infection of RABV. In this study, to obtain insights into the mechanism, we compared the peripheral infections of two fixed RABV strains, Nishigahara and the derivative Ni-CE, which cause lethal and asymptomatic infections, respectively, in mice after intramuscular inoculation. Examination of a series of chimeric viruses harboring respective genes from Nishigahara in the genetic background of Ni-CE revealed that Nishigahara phosphoprotein (P) gene plays a major role in peripheral infection by enhancing viral neuroinvasiveness. Results of both in vivo and in vitro experiments strongly suggested that Nishigahara P gene, but not Ni-CE P gene, functions to support stable replication of the virus in muscle cells. Further investigation based on the finding that RABV phosphoprotein counteracts the host interferon (IFN) system demonstrated that Nishigahara P gene, but not Ni-CE P gene, functions to suppress expressions of the Ifn-β gene and IFN-stimulated genes such as Mx1 and Oas1. In conclusion, we provide the first data strongly suggesting that RABV phosphoprotein supports stable viral replication in muscle cells by counteracting the host IFN system and, consequently, facilitates viral invasion into peripheral nerves.

245 **Measles virus inclusion bodies may be implicated in development of myopathy**

Hyun-Jeong Kwon1, Tomoyuki Honda1, Aya Nambu2, Tetsuro Arai1, Atsushi Miyakawa1, Hiroki Sato1, Susumu Nakae2, Misako Yoneda1, Chieko Kai1
1 Laboratory Animal Research Center, Institute of Medical Science, The University of Tokyo.
2 Laboratory of Systems Biology, Institute of Medical Science, The University of Tokyo.

Measles virus (MV) forms inclusion bodies in infected tissues regardless of the existence of infectious virion. Main component of the inclusion body is the nucleocapsid protein of MV (MV-N), the most abundant viral protein in infected cells. Although MV inclusion bodies are frequently observed in various tissues histopathologically after severe infection of MV, the function of them is still unknown. We generated transgenic mice expressing MV-N (MV-N mice) ubiquitously to assess pathogenic functions of MV-induced inclusion body. Initial growth of all F0 MV-N mice was normal, but two out of four F0 MV-N mice began to show weight loss and wasting at ~8 weeks of age and died at 15~17 weeks of age. Degeneration of muscle tissues was histopathologically observed in symptomatic MV-N mice but not in non-symptomatic MV-N mice and wild-type mice. Histochemically, accumulation of MV-N in muscle tissues was also well observed. The degenerations seen in the transgenic mice had similar characteristics to hereditary inclusion body myopathy(h-IBM). For investigating the influence of MV-N in regulation of muscle cell differentiation, C2C12 cells were either transfected with MV-N or infected with MV. Myogenic differentiation of the C2C12 cells either expressing MV-N or infected with MV were suppressed. Degenerations of muscle tissues seen in the MV-N expressing transgenic mice and inhibition of myogenic differentiation in C2C12 cells by MV-N indicate the relevance of MV-N to myopathy. These in vivo and in vitro models should be useful tools to study the pathogenic mechanism of h-IBM.

246 **Single Mutations in PB2 and NP Mediate Enhanced Pathogenicity of 2009 Pandemic H1N1 Influenza A Viruses in Mice**

Anna Otte (1), Martina Sauter (2), Karin Klingel (2) and Gülsah Gabriel (1)
(1) Heinrich Pette Institute, Leibniz Institute for Experimental Virology, Germany;
(2) Department for Molecular Pathology, Institute of Pathology, University Hospital Tübingen, Germany

Determinants of 2009 H1N1 influenza A virus pathogenicity are distinct from those known from studies with H5N1 or H7N7 highly pathogenic avian influenza A viruses (HPAIV). In order to determine novel molecular hallmarks of 2009 pandemic H1N1 pathogenicity, we have compared the virulence of two clinical isolates (designated as HH05 and HH15) representing early and late pandemic phase strains in mice. We found that HH15 is more virulent (logMLD50: 3.5 p.f.u.) than HH05 (logMLD50: >5 p.f.u.) in C57BL/6J mice. HH05 differs from HH15 by 12 amino acid substitutions in the PB2, PA, NP, HA, NA and NS1 genes. In order to address the molecular basis responsible for enhanced 2009 pandemic H1N1 virulence in mice, we have generated several recombinant influenza viruses by reverse genetics. Here, we have identified single mutations in the viral nucleoprotein (NP) and in the hemagglutinin (HA) which mediate enhanced pathogenicity in mice. Interestingly, these HH15-specific positions in HA, NA, NP and NS1 were less prevalent in the early pandemic periods while their prevalence increased during the late stages of the pandemic. Taken together, our findings reveal novel determinants of 2009 pandemic H1N1 pathogenicity distinct from those described for HPAIV isolated from humans. Furthermore, high prevalence of these positions in currently circulating strains suggests that these sites were replaced during later phases of the pandemic reflecting their selective advantage in the human population.

247 **Protection from RVFV neurologic disease is dependent on a functional CD4+ T cell Response**

Kimberly A. Dodd, Anita K. McElroy, Megan E. B. Jones, Stuart T. Nichol, and Christina F. Spiropoulou
1. US Centers for Disease Control and Prevention, Viral Special Pathogens Branch (all authors)
2. University of California Davis, School of Veterinary Medicine (Dodd)
3. Emory University School of Medicine, Division of Pediatric Infectious Disease (McElroy)
4. University of Georgia, College of Veterinary Medicine (Jones)

Rift Valley fever virus (RVFV) is an important public health pathogen that causes serious human disease, and in some cases, progresses to fatal hepatitis, a hemorrhagic syndrome or delayed onset-encephalitis. Little is known about the individual host characteristics and immune responses that predispose development of severe clinical disease. Here, we evaluated the role of T cells in primary RVFV infection using an established C57BL/6 mouse model. Due to the rapidly fatal disease course of wild-type RVFV in this model, we used attenuated ΔNSs RVFV for infection, which had the added benefit of permitting the adaptive responses to be evaluated in the context of an active innate response. Targeted T cell depletions demonstrated that CD4+ T cells, but not CD8+ T cells, were critical for controlling RVFV infection and preventing subsequent RVFV neurologic disease. CD4-depleted mice failed to clear ΔNSs virus from infected tissues and a third of these mice went on to develop delayed-onset encephalitis. We show that CD4+ T cells were required for the robust IgG and neutralizing antibody responses that correlated with clearance of RVFV from peripheral tissues in mock-depleted mice. Further, CD4-depleted mice demonstrated strong pro-inflammatory responses in the draining lymph node following ΔNSs infection, suggesting CD4+ T cells regulate the immune response in RVFV infection. These results indicate CD4+ T cells play a critical role in preventing RVFV pathogenesis and the development of clinical disease.

248 **Given the high mortality rates and lack of therapeutic treatments associated with the infections they cause, filoviruses are classified as BSL-4 agents.**

1Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, 575 Science Drive, Madison, WI 53711, USA
1Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, 575 Science Drive, Madison, WI 53711, USA
2Division of Gene Regulation, Institute for Advanced Medical Research, School of Medicine, Keio University, 35 Shinano-machi, Shinjuku-ku, Tokyo 160-8582, Japan
3Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan
4Department of Special Pathogens, International Research Center for InfectiousDiseases, Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo 108-22 8639, Japan

Given the high mortality rates and lack of therapeutic treatments associated with the infections they cause, filoviruses are classified as BSL-4 agents. This designation requires the highest level of laboratory biocontainment, which hampers research effortswith authentic filoviruses given the limited number of high biocontainment laboratories, experienced scientists, and specialized equipment for such tasks. Therefore, we established a biologically contained Ebola virus, EbolaΔVP30 virus. This virus lacks the essential viral VP30 gene and can only be maintained in cells that stably express this gene. EbolaΔVP30 virus is a BSL-3 agent that grows with similar kinetics and titers to those of authentic Ebola virus. A variant of the EbolaΔVP30 virus contains the reporter gene renilla luciferase (ren-luc), making it a useful and efficient tool for high-throughput screens. Suppression of ren-luc expression in the presence of inhibitor compounds is a reliable and robust read-out for high-throughput screens of small molecule compound libraries.This system can thus be used to efficiently identify inhibitors of any step in the virus life cycle, a clear advantage over single-step assays.

249 **A replication-incompetent influenza virus possessing PspA gene protects mice from influenza virus and S, pneumoniae infection.**

Hiroaki Katsura1, Zhenyu Piao2, Kiyoko Iwatsuki-Horimoto1, Yukihiro Akeda2, Kazunori Oishi2, Yoshihiro Kawaoka1,3,4
1Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan
2Laboratory of Clinical Research on Infectious Diseases, International Research Center for Infectious Diseases, Research Institute for Microbial Disease, Osaka University, Osaka 565-0871, Japan
3ERATO Infection-Induced Host Responses Project, Japan Science and Technology agency, Saitama 332-0012, Japan
4Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI 53706, USA

Streptococcus pneumoniae is a major causative pathogen in community-acquired pneumonia; together with influenza virus, it represents an important public health burden. Although vaccination is the most effective prophylaxis against these infectious agents, no vaccine simultaneously provides protective immunity against both S. pneumoniae and influenza virus. Here, to develop a bivalent vaccine that confers simultaneous protective immunity against both influenza virus and S. pneumoniae, we generated a replication-incompetent hemagglutinin-knockout (HA-KO) influenza virus possessing the sequence for the antigenic region of the pneumococcal surface protein A (PspA). Although this virus (HA-KO PspA virus) could replicate only in an HA-expressing cell line, it could infect individual wild-type cells (non-HA expressing cells) and express both viral proteins and PspA. PspA- and influenza virus-specific antibodies were elicited in nasal wash, bronchoalveolar lavage, and serum from mice intranasally inoculated with HA-KO PspA virus, and mice inoculated with HA-KO PspA virus were completely protected from lethal challenge with either S. pneumoniae or influenza virus. Further, bacterial colonization of the nasopharynx was prevented in mice inoculated with HA-KO PspA virus. These results indicate that HA-KO PspA virus is a promising bivalent vaccine candidate, and that this strategy can be a platform for the development of bivalent vaccine, based on replication-incompetent influenza virus, against other pathogens that cause respiratory infectious diseases.

250 **Recombinant influenza virus expressing the F protein of respiratory syncytial virus as a bivalent vaccine**

Wendy Fonseca 1*, Makoto Ozawa 2,3,4,Masato Hatta 2, Esther Orozco 1,5, Máximo B Martínez 5,6, Ramón Ocádiz-Ruiz 1, Yoshihiro Kawaoka 2,3,7,8.
1Departamento de Infectómica y Patogénesis Molecular, Centro de Investigación y de Estudios Avanzados, Instituto Politécnico Nacional, Mexico City, Mexico.
2Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI, USA.
3Department of Special Pathogens, International Research Center for Infectious Diseases, Institute of Medical Science, University of Tokyo, Shirokanedai, Minato-ku, Tokyo, Japan.
4Laboratory of Animal Hygiene, Joint Faculty of Veterinary Medicine, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan.
5 Universidad Autónoma de la Ciudad de México. Mexico City,Mexico.
6Centro de Diagnóstico y Vigilancia epidemiológica del Distrito Federal. Mexico City,Mexico.
7ERATO Infection-Induced Host Responses Project, Japan Science and Technology Agency, Saitama, Japan.
8Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, Shirokanedai, Minato-ku, Tokyo, Japan.

Influenza and respiratory syncytial virus (RSV) disease rank high among human respiratory diseases worldwide. In contrast to influenza, effective vaccine against RSV infection is not available: although formalin-inactivated RSV had been used in the 1960s, the vaccination enhanced, rather than reduced, the respiratory disorder. Previously, we developed a replication-incompetent influenza virus by replacing the coding sequence of the PB2 gene (viral RNA polymerase subunits) with that of a reporter gene. We also demonstrated the vaccine efficacy of this PB2-knockout (PB2-KO) virus against Influenza virus infection in a mouse model: the recombinant virus had even elicited the reporter protein-specific antibody. Here, we generated a PB2-KO recombinant influenza virus expressing the F protein of RSV (PB2-RSVF virus) and tested its potential as a bivalent vaccine against influenza virus and RSV infections. The PB2-RSVF virus stably expressed the RSV F protein in vitro, although its replication was restricted in PB2 protein-expressing cells. In mice intranasally immunized with the PB2-RSVF virus, efficient production of antibodies against influenza virus, but not RSV, were detected. We also demonstrated that PB2-RSVF virus-immunized mice were protected from a lethal challenge with influenza viruses. By contrast, RSV challenge of PB2-RSVF virus-immunized mice resulted in severe body weight loss, indicating that PB2-RSVF vaccination enhanced RSV-associated disease. The severity of the enhanced respiratory disease was not correlated with the level of the elicited RSV-specific antibody or RSV replication in lungs. These results indicate that the PB2-KO influenza virus-based vaccine is efficacious for influenza virus, but not for RSV infection.

251 **A protective and safe intranasal RSV vaccine based on a recombinant prefusion form of the F protein bound to a bacterium-like pa**

Alan Rigter,1,2, Ivy Widjaja,1,2, Hanneke Versantvoort,1, Maarten van Roosmalen,1, Kees Leenhouts,1, Peter J.M. Rottier,2, Bert Jan Haijema,1 and Cornelis A.M. de Haan,2
1 Mucosis B.V., Meditech Center, L.J. Zielstraweg 1, 9713 GX Groningen, The Netherlands. 2 Virology Division, Department of Infectious Diseases & Immunology, Faculty of Veterinary Medicine, Utrecht University, The Netherlands.

Respiratory syncytial virus (RSV) is an important cause of respiratory tract disease in infants and the elderly. Currently, no licensed vaccine against RSV is available. Here we describe the development of a safe and effective intranasal subunit vaccine that is based on recombinant fusion (F) protein bound to the surface of immunostimulatory bacterium-like particles (BLPs) derived from the food-grade bacterium Lactococcus lactis. Different variants of F were analyzed with respect to their conformation using neutralizing monoclonal antibodies, assuming that F proteins mimicking the metastable prefusion form of F expose a more extensive and relevant epitope repertoire than F proteins corresponding to the postfusion structure. Both addition of a trimerization motif and mutation of the furin cleavage sites increased the reactivity of F with the prefusion specific D25, with the highest reactivity being observed for F proteins in which both these features were combined. Intranasal vaccination of mice or cotton rats with BLPs loaded with this latter recombinant protein that mimics a prefusion form of F (BLP-F), resulted in the potent induction of F-specific immunoglobulins and in significantly decreased virus titers in the lungs upon RSV challenge. Moreover, and in contrast to animals vaccinated with formalin-inactivated RSV, animals that received BLP-F exhibited high levels of F-specific secretory IgA in the nose and RSV-neutralizing antibodies in sera, but did not show symptoms of enhanced disease after challenge with RSV.

252 **Guiding the immune response against influenza hemagglutinin towards the conserved stalk domain by altering glycosylation**

Dirk Eggink, Peter Palese
Department of Microbiology, Mount Sinai School of Medicine, New York, New York, USA

In general, the immune response against influenza vaccines focuses on the head domain of influenza hemagglutinin (HA) and neutralization is mostly caused by inhibition of receptor binding. However, the high variability in the head domain makes this immune response strain specific and antibodies show little to no cross-reactivity to drifted variants or strains from different subtypes. Recently, several antibodies have been described that target the stalk domain of HA and show broad reactivity within HA subtypes or even neutralize HAs of different subtypes. However, fewer of these broad reactive antibodies directed against the stalk domain are elicited upon vaccination. We try to focus the immune response towards the stalk domain and lower the immune response to the head domain, by introducing N-linked glycosylation sites in the head domain, covering the immuno dominant antigenic sites with glycans. At the same time we remove glycans of the stalk domain, thereby making it better accessible to the immune system. We are able to show that one can introduce up to eight additional glycosylation sites into the A/Puerto Rico/8/34 (PR8) head domain, resulting in the loss of binding of PR8 antiserum while keeping the ability to bind stalk directed antibodies. In parallel, two glycosylation sites in the stalk domain were removed, which did not affect protein expression and binding of several stalk directed antibodies, confirming proper folding of the stalk. These constructs are currently tested for their potential to elicit stalk directed neutralizing antibodies and provide broad protection in mice.

253 **Heterosubtypic antiviral activity of influenza virus hemagglutinin-specific antibodies**

Mieko Muramatsu, Reiko Yoshida, Ayato Takada
Division of Global Epidemiology, Hokkaido University Research Center for Zoonosis Control

Influenza A viruses of 16 hemagglutinin (HA; H1-H16) and 9 neuraminidase subtypes are maintained in aquatic birds. Of these, viruses of H1, H2, and H3 HA subtypes caused pandemics in humans in the last century, whereas direct avian-to-human transmission of avian influenza viruses which are antigenically different from these subtypes has been frequently reported with a public health concern. Influenza virus HA subtypes are principally defined as serotypes determined by the absence of cross-neutralization or -hemagglutination inhibition with polyclonal antisera to the respective HA subtypes. Thus, it is generally believed that neutralizing antibodies are not broadly cross-reactive among HA subtypes. We generated a novel monoclonal antibody (MAb) specific to HA, designated MAb S139/1, which showed heterosubtypic neutralizing and hemagglutination-inhibition activities against viruses of H1, H2, H3, and H13 subtypes. This MAb bound to a conformational epitope adjacent to the HA receptor-binding domain. On the other hand, we found that intranasal immunization of mice induced local and systemic IgA and IgG antibody responses to multiple HAs of different subtypes, while the induced antibodies showed no heterosubtypic neutralizing activity in a standard neutralization test in which viruses were mixed with antibodies prior to inoculation into cultured cells. Interestingly, however, a remarkable reduction of plaque formation and extracellular release of the heterologous virus was observed when infected cells were subsequently cultured in the presence of HA-specific cross-reactive IgA but not IgG antibodies. These data support the notion that antibodies play an important role in heterosubtypic immunity against influenza virus infection.

254 **M2e-immunity provides heterosubtypic protection and allows the induction of robust cross-protective T cell responses**

Michael Schotsaert1,2, Tine Ysenbaert1,2, Katrijn Neyt1,3, Lorena I. Ibañez1,2, Pieter Bogaert1,2, Bert Schepens1,2, Bart Lambrecht1,3, Walter Fiers1,2 & Xavier Saelens1,2
1Department for Molecular Biomedical Research, VIB, 9000 Ghent, Belgium
2Department of Biomedical Molecular Biology, Ghent University, 9000 Ghent, Belgium
3Department of Respiratory Diseases, University Hospital Ghent, 9000 Ghent, Belgium

Licensed influenza vaccines induce strain-specific virus-neutralizing antibodies but hamper the induction of possibly cross-protective T-cell responses upon subsequent infection. In contrast immunity induced by the universal influenza A vaccine candidate M2e (extracellular domain of matrix protein 2) is non-neutralizing and broadly protective. We compared protection induced by M2e-displaying recombinant virus-like particles with that of a conventional whole inactivated virus (WIV) vaccine using single as well as consecutive homo- and heterosubtypic challenges. Both vaccines protected against a primary challenge with a virus strain that matched the WIV vaccine. Functional T-cell responses were induced after primary challenge of M2e-immune mice but were absent in WIV-vaccinated mice. M2e-immune mice displayed limited inducible bronchus-associated lymphoid tissue (iBALT) which was absent in WIV-immune animals. Importantly, M2e- but not WIV-immune mice were protected from a primary as well as a secondary, severe heterosubtypic challenge, including challenge with pandemic H1N1 2009 virus. The combined immune response induced by M2e-vaccine and by clinically controlled influenza virus replication resulted in strong and broad protection against pandemic influenza. Our findings have important implications for influenza vaccination practices that focus on immunologically naive individuals, such as young children. We conclude that challenge of the M2e-immune host induces strong and broadly reactive immunity against influenza virus infection.

255 **What do we know about the 2009 H1N1 pandemic influenza virus and vaccine?**

Z. Chen, C. Cotter, AL Suguitan, X. Cheng, W. Wang, Q. Xu, J. Lu, S. Jacobson, S. Gee, R. Broome and H. Jin *MedImmune*

The 2009 H1N1 pandemic presented great challenges for rapid delivery of the H1N1pdm influenza vaccine. The issues included low vaccine yield, resistance to bromelain cleavage for HA antigen production and lower vaccine thermal stability. We have since solved these problems. We identified several key amino acids (119, 125, 127, 186) in the HA and one residue in the NA (369) that could improve vaccine virus yield without affecting antigenicity. We found that the E374 (HA2 #47) residue in the stalk region rendered the HA insensitive to the bromelain cleavage and also contributed to a higher pH (5.4) fusion threshold compared to the viruses with K374. We proved that the inter-monomer polar interaction between the highly conserved E21 and K374 residues lowered pH (5.0) fusion threshold and conferred higher structural and thermal stability. Next, we evaluated the contribution of H1N1pdm-specific antibodies from natural infection or acquired maternally in preventing subsequent virus infection and on vaccine mediated immune responses in ferrets. In addition, using newly developed ferret cellular immune reagents and assays, we showed that the live attenuated influenza vaccine containing the H1N1pdm vaccine component not only elicited stronger influenza-specific serum antibody responses but also T cell responses than the inactivated vaccine, in both naïve and influenza-seropositive animals. The H1N1pdm vaccine also offered significant protection against a heterologous seasonal H1N1 virus challenge infection in the upper respiratory tract of ferrets.

256 **Pseudotyped Newcastle Disease Virus with Paramyxovirus 8 Surface Glycoproteins and Highly Pathogenic Avian Influenza Virus HA**

Constanze Steglich1; Christian Grund2; Kristina Ramp1; Angele Breithaupt3; Jutta Veits1; Dirk Höper2; Günter Keil1; Mario Ziller ; Harald Granzow4; Thomas C. Mettenleiter1 and Angela Römer-Oberdörfer1¶

1Institute of Molecular Biology; 2Institute of Diagnostic Virology; 3Department of Experimental Animal Facilities and Biorisk Management, 4Institute of Infectology, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Südufer 10, D-17493 Greifswald-Insel Riems, Germany

The capability of Newcastle disease virus (NDV) as a vaccine vector virus which conveys protection against highly pathogenic avian influenza virus (HPAIV) has already been shown. However, pre-existing NDV antibodies may impair vector virus replication, resulting in a lower immune response also against the foreign protein additionally expressed, like HPAIV hemagglutinin. The development of a pseudotyped NDV which possesses functional surface glycoproteins different from NDV could overcome this problem. Here, we describe the construction of a pseudotyped vector NDV (pNDV-FHNPMV8H5) which carries the fusion protein (F) as well as the hemagglutinin-neuraminidase protein (HN) of avian paramyxovirus type 8 (APMV-8) instead of the corresponding NDV proteins. Additionally, the HPAIV H5 gene was inserted between the APMV-8 F- and HN-genes in a NDV backbone derived from the lentogenic NDV strain Clone 30 as already described for other NDV/AIV recombinants. After successful virus rescue, the resulting pNDVFHNPMV8H5 was further characterized. The expression of all three foreign genes was verified by Western blot analyses and indirect immunofluorescence. Furthermore, it could be shown that pNDVFHNPMV8H5 replicates comparably to the parental viruses, resulting in high titers in vitro and in vivo after 96 hours. Animal experiments were carried out to study the protection from a lethal HPAIV infection of SPF chicken without (MDA-) and with maternally derived NDV antibodies (MDA+) after immunization with pNDVFHNPMV8H5. MDA- as well as MDA+ chicken were protected from the lethal infection and virus shedding was significantly reduced.

257 **Live-attenuated respiratory syncytial virus vaccine candidates for clinical studies: Improved genetic stability and multiple mec**

Ursula J. Buchholz (1), Cindy Luongo (1), Christine C. Winter (1), Ruth A. Karron (2), Roderick S. Tang (3), Jeanne H. Schickli (3), and Peter L. Collins (1)
(1) RNA Viruses section, Laboratory of Infectious Diseases, NIAID, NIH, Bethesda, MD; (2) Center of Immunization Research, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; (3) MedImmune LLC, Gaithersburg, MD

Respiratory syncytial virus (RSV) is the leading cause of viral lower respiratory tract disease in infants and children worldwide. We previously developed a temperature-sensitive (ts) cDNA-derived virus named rA2cp248/404/1030ΔSH, in reference to its set of attenuating mutations, that is a promising vaccine candidate. In young infants, this virus was well-tolerated and immunogenic, but exhibited genetic instability in about one-third of post-vaccination nasal wash isolates, mostly due to reversion at the missense mutation "1030". This "1030" mutation is a single-nucleotide tyrosine-to-asparagine substitution at position 1321 of the L protein. We identified a reversion-resistant attenuating codon at position 1321 by inserting each of the 20 possible amino acids at position 1321 of wt rRSV. We confirmed the genetic stability of the alternative 1321 codon in in-vitro stress tests, but identified a second-site compensatory mutation at position 1313. By stabilizing the 1313 site as well, we generated a stable "1030" mutation. rA2cp248/404/1030ΔSH with stable "1030" mutation was attenuated in seronegative chimpanzees, and is being tested in a pediatric phase I study. Surprisingly, deletion of the 1313 codon yielded a viable virus with ts/attenuated phenotype. Combination with the previously tested NS2 gene deletion yielded a genetically stable virus with attenuation in chimpanzees comparable to rA2cp248/404/1030ΔSH, identifying this virus, designated as RSV ΔNS2 Δ1313 I1314L, as a second candidate for pediatric phase I studies. Thus, together with RSVΔM2-2 that is being tested in a phase I study, three genetically stable pediatric RSV vaccine candidates, each based on a different mode of attenuation, will be in clinical studies in 2013.

258 **Efficacy of a nonspreading Rift Valley fever virus expressing the Gn glycoprotein from the small genome segment**

Nadia Oreshkova, Rianka Vloet, Rob Moormann, Jeroen Kortekaas
Department of Virology, Central Veterinary Institute of Wageningen Univercity and Research Centre, P.O. Box 65, 8200 AB Lelystad, The Netherlands

Rift Valley fever virus (RVFV) is a mosquito-borne zoonotic bunyavirus of the genus Phlebovirus and a serious human and veterinary pathogen. The virus has a negative strand RNA, comprised of three segments, large (L), medium (M) and small (S). The proteins essential for genome replication are encoded by the L and S segments, whereas the M segment codes for the viral structural glycoproteins Gn and Gc. Recently we developed non-spreading RVFV particles (NSR) containing only the L and S segments. These particles are able to infect cells, replicate their genome and produce viral proteins, but are incapable of autonomous spread, therefore are called non-spreading RVFV (NSR). NSR was tested as a vaccine against RVFV in mice and sheep and showed promising efficacy. Here we report the expression of Gn, the major immunogenic protein of the virus, from the NSR small genome segment. The immunogenicity of the newly created particles, NSR-Gn, were compared with NSR in mice. NSR-Gn vaccination elicited superior CD8-restricted memory responses and higher virus neutralization titers. The vaccine was further characterized in lambs in a dose titration study. The highest vaccine dose, 106.3 TCID50/ml, protected all lambs from clinical signs and detectable viremia. The vaccinated lambs developed neutralizing antibodies within three weeks after vaccination and no boosts in the titers were observed upon challenge. These data suggest that sterilizing immunity was achieved by a single vaccination and shows that NSR-Gn optimally combines the efficacy of live vaccines with the safety of inactivated RVFV vaccines.

259 **Vesicular stomatitis virus-based vaccines protect Syrian hamsters from lethal Nipah virus challenge**

Blair L. DeBuysscher1, Joseph Prescott, Heinz Feldmann
1 Laboratory of Virology, DIR, NIAID, NIH, Hamilton, MT. 2 Division of Biological Sciences, University of Montana, Missoula, MT.

Nipah virus (NiV) is an emerging highly pathogenic zoonotic paramyxovirus with a broad species tropism. Outbreaks in humans have been occurring on an almost annual basis since 1998 in Malaysia, India or Bangladesh. Case fatality rates have been as high as 100% during outbreaks with clinical manifestations including acute encephalitis and/or respiratory distress. Currently, there are no approved vaccines or treatments for NiV infection. We have developed recombinant replication-competent vesicular stomatitis virus (VSV)-based vaccines, which lack the VSV glycoprotein, but instead encode an Ebolavirus glycoprotein for entry, as well as a NiV antigen. We have created three vaccine vectors that express either the fusion protein (F), glycoprotein (G), or nucleoprotein (N). These vaccines were tested in Syrian hamsters, which are the only small rodent model that closely mimics both the respiratory as well as encephalitic aspects of human disease. Animals were administered 105 plaque forming units of either vaccine and challenged 28 days later. At the time of challenge, all vaccinated animals had a measureable antibody response against NiV. In organs sampled, vaccinated animals euthanized five days post-challenge had drastically reduced levels of viral RNA compared to controls. Animals vaccinated with either glycoprotein were completely protected from disease. Hamsters vaccinated with the nucleoprotein showed increased survival and a delayed time to death compared to unvaccinated controls. Vaccination with VSV-NiV-based vaccines elicited a CD4+ and CD8+ T cell response. This study is the first to demonstrate that replication-competent VSV-NiV-based vaccines protected against NiV disease.

260 **Respiratory vaccination with live-attenuated measles virus: studies towards identification of the optimal site of delivery**

Rik de Swart
Erasmus MC, department Viroscience

The standard route of measles virus (MV) vaccination is subcutaneous injection, which is far from the natural route of entry. Programs evaluating alternative vaccination routes have been hampered by a lack of knowledge about the primary cells that should be targeted. The aim of our study was to determine whether MV vaccination via the respiratory route should target the upper or lower respiratory tract. We generated a recombinant (r) virus based on the Edmonston-Zagreb (EZ) strain, expressing enhanced green fluorescent protein (EGFP) from an additional transcription unit in position 3 of the genome. The virus was grown in MRC-5 cells and formulated with the same stabilizers and excipients used by the Serum Institute of India MVEZ vaccine. Four groups of twelve macaques were immunized with a dose of 104 TCID50 of rMVEZEGFP(3) via different routes of administration: injection, intra-tracheal inoculation, intra-nasal instillation or aerosol inhalation. In each group six animals were euthanized at early time points, whereas the other six were followed up to assess immunogenicity. Infected cells were detected in the muscle, nose and lungs, but systemic MV replication was virtually absent. Macrophages and dendritic cells appeared to be the predominant target cells in all cases. Animals vaccinated via the respiratory route had the highest specific serum antibody when the virus was delivered to the LRT. This study sheds new light on the tropism of live-attenuated MV vaccine, and identifies the lower respiratory tract as the optimal target when MV vaccine is delivered to the respiratory tract.

261 **Exploring host targets for broad-spectrum antivirals against respiratory viruses**

Daniel J. Chin (1), Cristian Cillóniz (2), Jade Carter (3), Gong Chen (1), Grant Beyleveld (4), Juan Ayllon (4), Peter Palese (4), Adolfo García-Sastre (4), Megan L. Shaw (4)
1. Translational Technology and Bioinformatics, Hoffman La-Roche, Nutley NJ 07110, USA
2. Virology Discovery Group, Hoffman La-Roche, Nutley NJ 07110, USA
3. Translational Research Sciences, Genetics and Genomics, Hoffman La-Roche, Nutley NJ 07110, USA
4. Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York NY 10029, USA

To identify host targets for a new generation of influenza antiviral therapy, several RNA interference (RNAi) screens have been performed to uncover required host factors. In total these screens have implicated over 1000 human proteins but there is minimal overlap between the screens and greater evidence for the vital role of a particular host factor is required to support its selection as a drug target. To this end we have employed an integrated "OMIC" approach to prioritize host factors with the most support for a role during influenza virus infection. Moreover, we have incorporated data on respiratory syncytial virus (RSV). Although these two viruses have distinct genetic structures, they encounter a similar host factor repertoire in the human respiratory tract and therefore they likely share dependency on many cellular functions. We have conducted RNA-seq experiments in parallel with influenza A virus and RSV to compare the profile of regulated host genes and then integrated these data with publicly-available datasets for virus-host (and host-host) protein interactions, influenza virus and RSV microarray data, and the influenza RNAi studies. Using two complementary bioinformatic strategies the data were filtered to reveal a subset of 53 host factors that are predicted to participate in the virus-host networks for both influenza virus and RSV. Inclusion of published data on quantitative protein expression in influenza virus or RSV infected cells reduces this further to 14 factors. We believe that this strategy greatly facilitates the identification of host factors to explore as targets for broad-spectrum antiviral drugs.

262 **MAP kinases and influenza virus infection - Inhibition of p38 MAP kinase protects mice from lethal H5N1 infection**

Ivonne Boergeling, Mirco Schmolke, Katharina Koether, Christina Ehrhardt, Stephan Ludwig
Institute of Molecular Virology, Center of Molecular Biology of Inflammation, University of Muenster, Germany

MAP kinases are major signal transducers in the cell. All four so far known MAP kinases (ERKs, JNKs, p38 MAPKs, ERK5) are activated by influenza A virus (IAV) infection. While the JNK MAPK pathway promotes the antiviral cytokine response, we have shown previously that the MEK/ERK MAPK pathway is exploited by IAV to support viral RNP export. Accordingly, we have demonstrated that inhibition of this signaling cascade blocks virus replication in vitro and in the animal model, representing a suitable target for a novel antiviral strategy. We now have investigated the role of p38 MAPK in the antiviral response against IAV in cells and animals. Global gene expression profiling of HPAIV infected cells in the presence of the p38 inhibitor SB202190 revealed, that inhibition of p38 leads to reduced expression of type I interferons (IFN) and other cytokines after H5N1 and H7N7 infection. More than 90% of all virus-induced genes were either partially or fully dependent on p38. This could be attributed to the fact that p38 inhibition not only affects primary gene expression responses to infection but also impairs the secondary gene expression response by interference with the JAK/STAT pathway. *In vivo* inhibition of p38 leads to a nearly complete shutdown of virus induced cytokine expression concomitant with reduced viral titers, thereby protecting mice from lethal H5N1 IAV infection. These observations show, that p38 acts on two levels of the antiviral IFN response: Initially the kinase regulates IFN induction and later p38 controls IFN signaling by STAT phosphorylation and thereby promotes expression of IFN-stimulated genes. Thus, inhibition of p38 maybe an antiviral strategy that protects mice from lethal influenza via suppression of overshooting cytokine expression.

263 **Hemagglutinin activating host cell proteases provide promising drug targets for the treatment of influenza virus infections**

Eva Böttcher-Friebertshäuser (1), Catharina Peitsch (1), Carolin Tarnow (1), Daniela Meyer (2), Torsten Steinmetzer (2), Hans-Dieter Klenk (1), Wolfgang Garten (1)

(1) Institute of Virology, Philipps-University Marburg, Germany

(2) Institute of Pharmaceutical Chemistry, Philipps-University Marburg, Germany

Influenza is a highly contagious acute infection of the respiratory tract that affects millions of people each year. Current measures for the control of influenza are vaccination and antiviral medications, which target the viral neuraminidase (NA) or the M2 protein. The development of drug resistance for both M2 and NA inhibitors, however, highlights the need for novel drug targets. Cleavage of the surface glycoprotein hemagglutinin (HA) by host cell proteases is crucial for virus infectivity and, hence, relevant proteases provide potential drug targets. Human influenza viruses are activated at a monobasic HA cleavage site and we identified HAT (human airway trypsin-like protease) and TMPRSS2 as relevant HA-activating proteases in the airways. Here, we investigated the efficacy of two structurally different serine protease inhibitors to prevent influenza virus multicycle replication by inhibition of HA cleavage. Benzylsulfonyl-d-arginine-proline-4-amidinobenzylamide (BAPA) is a potent inhibitor of HAT and TMPRSS2, whereas the 3-amidinophenylalanine derivative MI-432 only inhibits TMPRSS2. Both inhibitors efficiently suppressed virus propagation in TMPRSS2-expressing human airway epithelial cells. Inhibitor treatment reduced virus titres of different influenza A and B viruses more than 1000fold and delayed virus propagation by 24-48 hours at non-cytotoxic concentrations. Interestingly, the combination of BAPA or MI-432 with the NA inhibitor oseltamivir carboxylate was highly synergistic and blocked influenza virus propagation in airway epithelial cells at remarkably lower inhibitor concentrations than treatment with either inhibitor alone. Treatment of experimentally infected mice is currently under investigation. Our studies show that protease inhibition provides a promising approach that should be considered for influenza treatment.

264 **A cell-based screening system for influenza A viral RNA transcription/replication Inhibitors**

Makoto Ozawa1,2,3, Masayuki Shimojima4,5, Hideo Goto4,6, Shinji Watanabe2,7, Yasuko Hatta2,8, Maki Kiso4, Yousuke Furuta9, Taisuke Horimoto4,10, Noel R. Peters11, F. Michael Hoffmann11, and Yoshihiro Kawaoka1,2,4,5

1Department of Special Pathogens, International Research Center for Infectious Diseases, Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan
2Influenza Research Institute, Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI 53711, USA
3Laboratory of Animal Hygiene, Joint Faculty of Veterinary Medicine, Kagoshima University, Kagoshima 890-0065, Japan
4Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan
5Laboratory of Veterinary Microbiology, Faculty of Agriculture, Yamaguchi University, Yamaguchi 753-8515, Japan
6Department of Microbiology, Wakayama Medical University, Wakayama, 641-8509, Japan
7ERATO Infection-Induced Host Responses Project, Saitama 332-0012, Japan
8FluGen, Inc. 545 Science Drive, Madison, WI 53711, USA
9Toyama Chemical Co., Ltd., Toyama 930-8508, Japan
10Department of Veterinary Microbiology, Graduate School of Agricultural and Life Sciences, University of Tokyo, Tokyo 113-8657, Japan.
11UW Small Molecule Screening Facility and Medicinal Chemistry Core Facility, University of Wisconsin-Madison, Madison, WI 53792, USA

Although two classes of antivirals, NA inhibitors and M2 ion channel blockers, are licensed for influenza treatment, dual resistant mutants, including highly pathogenic H5N1 viruses, have appeared. Alternative treatment options are, therefore, needed. Influenza A viral RNA (vRNA) transcription/replication is a promising target for antiviral development, since it is essential for virus replication. Accordingly, an efficient and reliable method to identify vRNA transcription/replication inhibitors is desirable. Here, we developed a cell-based screening system by establishing a cell line that stably expresses influenza viral ribonucleoprotein complex (vRNP). Compound library screening using this cell line allowed us to identify a compound that inhibits vRNA transcription/replication by using reporter protein expression from virus-like RNA as a readout and virus replication in vitro. vRNP-expressing cells have potential as a simple and convenient high-throughput screening (HTS) system, and, thus, are promising to identify vRNA transcription/replication inhibitors for various RNA viruses, especially for primary screens.

265 **Mechanism-Based Covalent Inhibitors of Influenza Virus Neuraminidases show broad spectrum antiviral efficacy in vitro and in viv**

Jennifer L. McKimm-Breschkin1, Susan Barrett1, Victor A. Streltsov1, Pat Pilling1, Jin-Hyo Kim2, Ricardo Resende2, Tom Wennekes2, Hong-Ming Chen2, Nicole Bance3, Sabrina Buchini2, Andrew G. Watts4, Martin Petric5, Richard Liggins6, Masahiro Niikura3 and Stephen G.Withers1

1CSIRO Materials Science and Engineering, 343 Royal Pde, Parkville, 3052, Australia.

2Department of Chemistry, University of British Columbia, 2036 Main Mall, Vancouver, B.C., V6T1Z1, Canada.

3Faculty of Health Sciences, Simon Fraser University, 8888 University Drive, Burnaby, B.C., V5A1S6, Canada.

4Department of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath, BA27AY, UK.

5British Columbia Centre for Disease Control, Provincial Health Services Authority, Vancouver, BC, V5Z 4R4, Canada.

6Centre for Drug Research and Development (CDRD), 2259 Lower Mall, Vancouver, B.C., V6T 1Z4, Canada.

While influenza vaccines play an important role in the prevention of disease, due to continual evolution of the viruses the strains in the seasonal vaccine may not match those circulating by the winter, nor do they provide 100% protection. Furthermore in the event of a new pandemic strain, it still takes months to prepare the new vaccines. Hence antivirals can play an important role in modulating severity of the influenza infection when vaccines are not available. Both Tamiflu and Relenza are licensed globally for the treatment and prevention of influenza, however we are now seeing resistance to Tamiflu, and some of these mutations are causing cross-resistance to Relenza. We describe here a new class of potent, specific mechanism-based inhibitors of the influenza neuraminidase. They are based on difluorosialic acid (DFSA) and function by the transient formation of a covalent intermediate species, forming a link to Tyr406 in the enzyme active site, demonstrated enzymatically and by structural analysis. We have demonstrated efficacy in enzyme inhibition assays *in vitro* and in cell-based plaque reduction assays, with IC50s in the low nM range. Importantly we have demonstrated broad spectrum efficacy against viruses resistant to Tamiflu and Relenza, thus indicating a different resistance profile. The DFSAs were also effective in protecting mice against a lethal infection with influenza at drug concentrations comparable to Relenza. Based on the similarity of their structure to that of the natural substrate and their mechanism-based design we predict resistance is less likely to arise.

266 **A cell-based screening system to evaluate the susceptibility of influenza viruses to T-705 (favipiravir)**

Emi Takashita, Miho Ejima, Seiichiro Fujisaki, Noriko Kishida, Hong Xu, Masaki Imai, Masato Tashiro, Takato Odagiri
Influenza Virus Research Center, National Institute of Infectious Diseases, Tokyo, Japan

In Japan, four neuraminidase (NA) inhibitors, oseltamivir, peramivir, zanamivir and laninamivir, are approved for chemotherapy against influenza and are prescribed with the highest frequency in the world. Therefore, Japan could be at high risk for the emergence and spread of antiviral-resistant viruses and a nationwide monitoring of resistant viruses is desired. To date a new drug application has been submitted in Japan for a novel antiviral compound T-705 (favipiravir), which targets the RNA-dependent RNA polymerase and inhibits the viral replication of influenza viruses. Here, we developed a cell-based screening system to evaluate the susceptibility of influenza viruses to T-705 for antiviral-resistance monitoring. Antiviral activity of T-705 was determined by inhibition of virus-induced cytopathic effect and the susceptibility of the viruses was expressed as 50% effective concentration (EC50). Using our system, we found that T-705 has antiviral activity against NA inhibitor-resistant A(H1N1)pdm09, A(H3N2) and B viruses, which possess an H275Y, E119V or D197E substitution in the NA protein, respectively. Next, we examined clinical isolates obtained from clinical trials of T-705. Pairs of A(H1N1)pdm09, A(H3N2) and B viruses isolated before and during administration of T-705 were analyzed and there was no virus with reduced susceptibility to T-705 during administration. We found that two of 20 paired A(H1N1)pdm09 viruses and one of 20 paired B viruses possessed a substitution in PB1, PB2 or PA protein during administration, but the susceptibility of the viruses to T-705 was not affected. This study was a collaboration with Dr Yousuke Furuta (Toyama Chemical Co., Ltd., Japan).

267 **New generation of fusion inhibitors against paramyxoviruses**

Cyrille Mathieu (1)*, Samantha G Palmer (1)*, Ilaria De Vito (1), Antonello Pessi (3), Stefan Niewisk (4), Branka Horvat (5), Matteo Porotto (1) and Anne Moscona (1,2)
Department of (1)Pediatrics and of (2)Microbiology ad Immunology, Weill Medical College of Cornell University, New York, NY10021.
(3)JV Bio and CEINGE, Via Gaetano Salvatore 486, 80145 Napoli (NA) Italy.
(4)Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210
(5)International Center for Research in Infectious Diseases -CIRI, INSERM U1111, CNRS UMR5308, ENS- Lyon, University of Lyon 1, 21 Avenue T. Garnier, 69007 Lyon, France

Enveloped viruses begin their life cycle with entry into their host target cells. For paramyxoviruses like human parainfluenza virus type 3 (HPIV3) or Nipah virus (NiV), the surface receptor-binding (HN or G) and fusion (F) glycoproteins mediate virion attachment to cells and fusion of the viral and cellular membranes. The HN/G receptor interaction activates the fusion protein via a series of conformational changes leading to the insertion of the fusion peptide into the target membrane. The class I fusion proteins share two heptad-repeat regions in each ectodomain which refold to form an antiparallel six-helix bundle structure allowing the membrane fusion. Peptides derived from the C-terminal repeat (HRC) can interfere with the formation of this six-helix bundle by binding to the transiently exposed N-terminal repeat, and thus block the fusion. The fusion inhibitors discussed here possess three functional groups: a peptide, a linker and a lipid moiety. This design improves penetration into the CNS. Here we show modifications in the amino acid sequence and the linker that considerably improve the effacy of the fusion inhibitors against NiV and HPIV3 both in vitro and in vivo. The in vivo efficacy of this new generation of lipid-tagged peptides suggests that they are promising candidates for preventing or treating infection by lethal paramyxoviruses.

268 **Development of a Robust RSV Replicon Assay for High-Throughput Screening**

Choi Lai Tiong-Yip, Helen Plant, Jun Fan, Paul Sharpe, Kirsty Rich, Elise Gorseth, Qin Yu
Infection Innovative Medicines Unit, AstraZeneca R&D Boston, 35 Gatehouse Drive, Waltham, Massachusetts 02451, United States; Discovery Sciences, AstraZeneca R&D Alderley Park, Macclesfield, Cheshire, England.

Respiratory syncytial virus (RSV) antiviral discovery has been hindered by the lack of lack of a robust and convenient assay for high-throughput screening (HTS) of large compound libraries. In this paper, we present the development and optimization of a 384-well RSV replicon assay that enabled HTS for RSV replication inhibitors with minimal biocontainment. 0.6 across 200 assay plates and a signal to background ratio of»Through the use of cryopreserved and enriched replicon cells, assay-ready compound plates, and other optimized assay conditions, the assay was successfully automated with significant improvement in assay signal, reproducibility, and throughput, with calculated Z' >40. A validation screen was performed with 7,000 compounds in duplicate and demonstrated high assay reproducibility. The dose response replicon assay was further validated with different classes of RSV replication inhibitors. The fully optimized RSV replicon assay has enabled multiple HTS campaigns for RSV replication inhibitors and demonstrated high hit confirmation rates. We further combined the RSV replicon and cytotoxicity assays into a novel multiplex assay that delivers RSV replicon and cytotoxicity readouts simultaneously. By taking advantage of the renilla luciferase reporter in RSV replicon which allows live-cell readout prior to measuring cytotoxicity, these two assays were successfully combined as a single assay. This provides a high-quality and cost-saving option to support the lead optimization following the HTS. This multiplex assay format is also translatable to streamline other antiviral assays. In summary, we have developed a robust RSV replicon assay which could help expedite the discovery of novel RSV therapeutics.

269 **Non-invasive real-time monitoring of Junín virus infection**

Nadezhda Yun, Alexey Seregin, Milagros Miller and Slobodan Paessler.
Department of Pathology, Galveston National Laboratory, University of Texas Medical Branch, Galveston, TX

Arenavirus Junín (JUNV) is an enveloped virus with a bisegmented negative-stranded RNA genome. JUNV causes Argentine hemorrhagic fever (AHF), a severe illness with hemorrhagic and neurological manifestations and a case fatality rate of up to 15-30%. In addition to its impact on public health, JUNV possesses features that make it suitable as a potential biological weapon. We have recently developed a cDNA-based reverse genetics system for a pathogenic Romero strain of JUNV (rRomero). Based on this system, we have generated plasmid constructs for the rescue of recombinant three-segmented Junin Romero viruses expressing either a luciferase reporter (Luc) (r3Romero-Luc) or green fluorescent protein (GFP) (r3Romero-GFP). Both three-segmented viruses exhibited a delayed growth in Vero cells. r3Romero-Luc and r3Romero-GFP viruses reached high titers; however, their growth was delayed by 24 and ~36 hours, respectively, compared to the parental bi-segmented rRomero virus. To characterize in vivo properties of the three-segmented JUNV we utilized our recently established alpha/beta and gamma interferon receptors deficient mouse model. These mice develop disseminated infection and a severe uniformly lethal disease after infection with JUNV Romero. In contrast, r3Romero-Luc exhibited an attenuated phenotype and we did not observe any disease symptoms in these animals. Replication of this virus was primarily restricted to the spleen and liver as detected by in-vivo imaging system (IVIS) and persisted for at least 14 days p.i. Thus, three-segmented JUNVs that we generated could be potentially used for pathogenesis studies, screening of antiviral compounds that directly target virus replication, and development of live-attenuated vaccines.

270 **Recombinant adenovirus-based DIVA vaccine protects against virulent peste des petits ruminants virus**

Becky Herbert, Jana Baron, Geraldine Taylor, Michael D Baron
The Pirbright Institute, Surrey UK

Peste des petits ruminants virus (PPRV) is a morbillivirus that can cause severe disease, characterised by pyrexia, pneumoenteritis, and gastritis in sheep and goats. The socio-economic burden of the disease is increasing in underdeveloped countries, with poor livestock keepers being affected the most. Current vaccines consist of cell-culture attenuated strains of PPRV, which induce similar antibody profiles to that induced by natural infection. Generation of a vaccine which enables differentiation of infected and vaccinated animals (DIVA) would be of huge benefit for eradication programmes. In an effort to create a vaccine that would enable infected animals to be distinguished from vaccinated ones (DIVA vaccine), we have evaluated the immunogenicity of recombinant fowlpox (rFPV) and recombinant human adenovirus (rAd5), expressing PPRV F and H proteins, in goats. The rAd5 constructs induced higher levels of virus-specific and neutralising antibodies, and primed greater numbers of CD8+ T cells than the rFPV-vectored vaccines in goats. A prolonged antibody response was observed in when the vaccine included rAd5 expressing ovine GM-CSF and IL-2. A single dose of rAd5-H, with or without the addition of the cytokine-expressing constructs, protected animals from challenge with virulent PPRV, 12 weeks after vaccination. These constructs offer the promise of DIVA vaccine capability coupled with greater thermotolerance than the existing vaccines.

271 **A Microparticle Vaccine Containing the CS3C motif of the RSV G Protein Induces Protection Against RSV Infection in Mice**

Patricia Jorquera 1, Katie Oakley 1, Thomas J. Powell 2, and Ralph A. Tripp 1
1 Department of Infectious Diseases, University of Georgia, 2 Artifical Cell Technologies Inc.

Respiratory syncytial virus (RSV) is the most important cause of serious lower respiratory tract illness in infants and the elderly worldwide. Currently, no safe and efficacious RSV vaccine exists. Progress in our understanding of RSV infection has shown that RSV G protein contains a CX3C chemokine motif that modifies the activity of fractalkine (CX3CL1) affecting immunity and disease pathogenesis. Previous work in our lab has shown that immunization of mice with the central conserved region of the RSV G protein generates antibodies that inhibit G protein CX3C-CX3CR1 interaction and reduce disease pathogenesis. In this study, BALB/c mice were vaccinated with microparticle vaccines produced by layer-by-layer assembly of RSV G protein polypeptides containing the CX3C motif (GA2-MP). The findings show that mice immunized with GA2-MP produced neutralizing antibody responses and strong T cell responses associated with reduced pulmonary virus replication following RSV challenge. Analysis of bronchioalveolar lavages demonstrated that upon RSV challenge mice vaccinated with GA2-MP develop a balance Th1/Th2 response and have increased levels of RSV M2-specific CD8+ T cells in the lungs compared to the FI-RSV vaccinated group. Importantly, vaccination was not associated with increased pulmonary eosinophilia following RSV challenge showing that vaccination with the RSV G microparticle vaccines is robust, safe and effective.

abstracts

272 **Prime-boost vaccination against highly pathogenic avian influenza H5N1 using a Newcastle disease virus vector in day-old chicks**

Helena Lage Ferreira1, Fabienne Rauw2, Jean François Pirlot2, Frédéric Reynard3, Thierry van den Berg2, Michel Bublot3, Bénédicte Lambrecht2
1 FZEA-USP, Av. Duque de Caxias Norte, 225, Pirassununga – SP, CEP 13635-900, Brazil
2 CODA-CERVA-VAR, Groeselenberg 99, B-1180 Uccle, Brussels, Belgium
3 MERIAL S.A.S., 254 rue Marcel Merieux, 69007 Lyon, France

Avian influenza (AI) vaccines should be used as part of a whole comprehensive AI control program. Vectored vaccines based on Newcastle disease virus (NDV) are very promising but are licensed in a few countries so far. In the present study, the immunogenicity and protection against a highly pathogenic (HP) H5N1 influenza challenge were evaluated after vaccination with an enterotropic NDV vector expressing an H5 hemagglutinin (rNDV-H5) in day-old SPF chickens inoculated once, twice or once followed by a heterologous boost with an inactivated H5N9 vaccine (iH5N9). The heterologous prime-boost rNDV-H5/iH5N9 combination afforded the best level of protection against the H5N1 challenge performed at 6 weeks of age and induced a broader detectable immunity including systemic, mucosal and cellular AI-specific responses. Two rNDV-H5 administrations conferred a good level of protection after challenge, although only a cellular H5-specific response could be detected. Interestingly, a single administration of rNDV-H5 gave the same level of protection as the double administration without any detectable H5-specific immune response detection. In contrast to AI immunity, humoral, mucosal and cellular NDV-specific immunity could be detected after 2, 2 and 4 weeks post vaccination, respectively. Both AI and NDV specific cellular immune responses were slightly higher after the double rNDV-H5 vaccination when compared to the single inoculation, Our results indicate that this enterotropic NDV vector vaccine could be suitable as a bivalent AI and ND vaccine administered in poultry at the hatchery.

273 **Tyrosine-Kinase Inhibitor (Vandetanib) reduces virus replication and increase survival in a Hantavirus Pulmonary Syndrome model**

Brian H. Bird1, Punya Shirvastava-Ranjan1, Bobbie Rae Erickson1, Kimberley A. Dodd1,2, Christina F. Spiropoulou1
1 Viral Special Pathogens Branch, Division of High Consequence Pathogens and Pathology, Centers for Disease Control and Prevention, Atlanta, GA 30333
2 University of California Davis School of Veterinary Medicine, Davis, CA 95616

Hantavirus pulmonary syndrome (HPS) is a severe disease associated with hantavirus infection. High level virus replication occurs in microvascular endothelial cells without pronounced virus-induced cytopathic effects. However, virus infection leads to pronounced microvascular leakage, accompanied by pulmonary and pleural edema and high case fatality. Previously, we demonstrated that infection with Andes virus (ANDV) caused upregulation of vascular endothelial growth factor (VEGF) and concomitant downregulation of the cellular adhesion molecule VE-cadherin leading to increased vascular permeability. Analyses of severe human HPS-patient sera further demonstrated increased circulating VEGF. To further investigate the role of VEGF on HPS pathogenesis, initial in vitro experiments using a VEGF-antagonist tyrosine-kinase inhibitor (vandetanib-VAN) in HMVEC-Lung cells were completed. Potent downregulation of both VEGFwhere is this comomg from? In vivo?and VEGFR-2 receptor phosphorylation following ANDV infection was found. In vivo, VAN treatment in a hamster model of HPS significantly improved survival (VAN 12/40; sham 0/10; p=0.005) and reduced mean pleural effusion volumes (VAN 1.1mL; sham 5.1mL). Serial euthanasia studies revealed an unexpected reduction in virus replication and IP-10 chemokine production among VAN treated animals at days 3 and 6 post-infection. Subsequent in vitro studies confirmed VAN reduction of ANDV G2 expression and virus growth and reduced cellular levels of the receptor protein (αβ3-Integrin). Thus limiting virus entry and spread via cellular mediated endocytosis. Taken together, these studies demonstrate that tyrosine-kinase inhibitors such as vandetanib can have pluripotent effects on both virus entry via receptor downregulation, and specific antagonism of a known pathogenesis mediator (VEGF) leading to modest but significant gains in survivability.

274 **Screening and optimization of Respiratory Syncytial Virus fusion inhibitors**

Vanessa Gaillard1, Julien Héritier1, Dominique Garcin2, Jean-Manuel Segura1, Elodie Baechler3, Marc
Mathieu1 and Origène Nyanguile1
1HES-SO Valais, Route du Rawyl 47, 1950 Sion, Switzerland
2University of Geneva, School of Medicine, 1 rue Michel Servet, 1211 Geneva, Switzerland
3Bio-Rad Laboratories, Diamed GmbH, Pra Rond 23, 1785 Cressier FR, Switzerland

Respiratory syncytial virus infections are one of the leading causes of mortality in infants worldwide and cause a significant hospitalization burden both in the US and in Europe. There is currently only one treatment available, a prophylactic antibody (Synagis/Palivizumab) that is administered only to at risk premature infants because of its highly prohibitive cost. Peptides derived from the heptad repeat (HR) region of the fusion protein F can inhibit virus cell entry into host cell. However, little effort has been devoted to develop such antivirals because of the relatively weak inhibitory activity of the native RSV peptides. This may be due to a poor intrinsic helical content of these peptides and perhaps also because of the kinetics of fusion, which has been showed to considerably differ among paramyxoviruses. Here, we wish to investigate if helix stabilization can increase the inhibitory activity of RSV derived HR peptides. We use the all-hydrocarbon stapled peptide technology with the aim to stabilize the alpha helical configuration of these peptides and improve their pharmacological properties. We have shortened the F HRB domain into three subdomains which are scanned for various stapling combinations. We will present the activity of the resulting peptides in a biochemical fluorescence polarization assay and in a cellular infection assay, as well as a preliminary investigation of the peptide helical content by circular dichroism.

275 **DNA vaccine provides solid protection in ducks against heterologous H5N1 Avian Influenza Virus challenge**

Junping Li, Shuangcheng Zhao, Guojun Wang, Jinxiong Liu, Pucheng chen, Jianzhong Shi, Xianying Zeng, Yanbing Li, Guohua Deng, Guobin Tian, Yongping Jiang and Hualan Chen

Ducks play an important role in maintenance of highly pathogenic H5N1 avian influenza viruses (AIV) in nature, and successful control of AIVs in ducks has important implications for eradication of the disease in poultry and its prevention in humans. DNA Vaccine pCAGGoptiHA, which expressing a codon-optimized hemagglutinin (HA) gene of H5N1 virus A/goose/Guangdong/1/1996 (GS/GD/96), induces strong immune response and protective efficacy in chickens and quail. In this study, we evaluated the immunogenicity and protective efficacy of pCAGGoptiHA in ducks. Ten 3-week-old specific pathogen free (SPF) ducks were inoculated with two doses of 30 μg pCAGGoptiHA and their sera were collected weekly for checking the hemagglutinin inhibition (HI) antibody duration. The HI titer reached to 6.8log2 one week after the second dose of inoculation and then maintained at an average of 4log2-6.3log2 during the next 27 weeks. For the challenge study, groups of eight 3-week-old SPF ducks were intramuscularly inoculated two times at 3-week intervals with 30 μg of the plasmid pCAGGoptiHA or PBS as control. The HI antibodies were monitored weekly. Two weeks after the second inoculation, the ducks were challenged with 105EID50 of heterologous highly pathogenic avian influenza virus A/duck/Hubei/49/2005(DK/HB/49) (clade 2.3.4), A/duck/Liaoning/51/2005 (DK/LN/51) (clade 2.2) and A/duck/Guangdong/322/2010 (DK/GD/322) (clade 2.3.2), respectively, by intranasally inoculation. Oropharyngeal and cloacal swabs were collected on days 3, 5 and 7 after inoculation, and ducks were observed daily for disease signs and deaths for two weeks. The HI antibody to the GS/GD/96 virus was detected in all vaccinated ducks after the first vaccination, and increased sharply after the second dose of inoculation. All of the vaccinated ducks were completely protected from the challenge of the DK/HB/49 and DK/LN/51, virus shedding, disease signs, and death were not detected from any birds. In the DK/GD/322 challenged group, one duck shed virus and died at 11 days post challenge. The PBS inoculated ducks died within 7 days after challenge with the H5N1 viruses. These results indicate that the plasmid pCAGGoptiHA can be a candidate vaccine for preventing AIV infection in ducks.

276

intranasal Vaccination with Integrase-Defective Lentiviral Vectors encoding Influenza Nucleoprotein Induce Protective Immunity t

J. Fontana1, Z. Michelini2, D. Negri2, A. Cara2, M. Salvatore1
1Weill Cornell Medical College, New York, US, 2Istituto Superiore di Sanita, Rome, ITALY

Vaccines based on highly conserved antigens can provide protection against different influenza A strains and subtypes. Influenza nucleoprotein (NP) is a prominent CD8+ and CD4+ T cell target of and is >90% conserved among influenza A isolates, so is of interest as vaccine candidates. In this study, we show that integrase-defective lentiviral vectors (ID-LV) express sustained levels of NP capable of inducing robust immune responses that can be exploited for vaccine development. Lentiviral vectors are good inducers of cellular and humoral immunity, but they carry the potential risk of integration into the host genome. Instead, ID-LV have a mutation in the integrase gene that prevents genomic integration. Therefore they are stably retained in the nucleus as episomal circular DNA that expresses functional proteins in the context of a non-replicating and non-integrating virus. ID-LV expressing NP (ID-LV-NP) were made by three plasmids co-transfection into 293T cells. NP expression in vitro was confirmed by flow cytometry and Western blot. Administration of ID-LV-NP induced persistent NP-specific CTL and serum antibody responses in mice, as measured by IFN-γ ELISPOT and ELISA assays. Mice receiving two intranasal doses of ID-LV-NP were fully protected against homologous and heterologous challenge with influenza A virus (10 LD50) and showed no sign of disease. Partial protection was seen after intramuscular immunization with ID-LV-NP following prime with NP-expressing DNA or ID-LV-NP, but mice exhibited marked weight loss. This study supports the potential use of ID-LV-based vaccines for protection against influenza virus infection, possibly due to induction of mucosal immunity.

277

278

Development of Recombinant Canine Distemper Virus (rCDV) Vectors for SIV/ HIV Vaccine Delivery

Xinsheng Zhang, Olivia Wallace, Kevin Wright, Palka Sharma, Ninkka Tamot, Mary Lopez, Arban Domi, John Coleman, Gavin Morrow, Christopher Parks
The AIDS Vaccine Design and Development Laboratory, International AIDS Vaccine Initiative

Live-attenuated viral vaccines have controlled infectious viral diseases like smallpox, yellow fever, polio, and measles. However, developing a live HIV vaccine is not practical due to the highly mutable nature of the virus. We aim to harness safe and efficacious live-attenuated morbilliviruses to develop vectored HIV vaccines. Replicating rCDV was chosen to utilize its lymphotropism to promote vaccine delivery to lymphoid tissues, and to minimize potential effects of pre-existing measles virus immunity. To use the SIV model, we have rescued rCDV vectors expressing soluble or membrane-anchored Envelope (Env) or Gag with and without its myristoylation signal. Ferrets or macaques vaccinated with the rCDV vectors experienced no adverse effects after intranasal instillation of 1 x 107 PFU. Vector replication and anti-SIV immune responses were observed in both species. In a small study, 2 macaques were primed intranasally with rCDV vectors encoding soluble Env and myristoylation-positive Gag after which they were boosted intramuscularly with Ad5-SIV vaccines. Following rCDV immunization, Env antibody titers were observed in serum, which were boosted by Ad5-Env immunization. Gag responses were relatively low. The animals were challenged with SIVmac239, and an approximate 2-log lower set-point plasma virus load was observed in the 2 macaques vaccinated with rCDV vectors and Ad5-SIV when compared to an animal that was vaccinated only with Ad5-SIV. These results demonstrated that intranasal rCDV vaccination was safe for macaques, the vectors were immunogenic, and immunization provided increased control over SIV replication; therefore, a larger study has commenced to evaluate protective immunity induced by rCDV vectors.

279

280 **Vaccination with recombinant PIV5 expressing influenza antigens provides diverse immunity and protection against homologous and**

S. Mark Tompkins, Alaina J. Mooney, Zhou Li, Jon D. Gabbard, Dan Dlugolenski, Scott Johnson, Biao He *Department of Infectious Diseases, University of Georgia, Athens, Georgia*

Vaccination is considered the most effective measure for controlling influenza virus, however current vaccination methods are inadequate. Virus-vectored vaccines offer an appealing alternative. Parainfluenza virus 5 (PIV5), a non-segmented, negative-stranded RNA virus in the family Paramyxoviridae is an attractive vector candidate as it has a stable genome without a DNA phase in its lifecycle, is readily grown to high titers in approved vaccine cell lines, and infects many mammals without causing disease. Influenza vaccines expressing variety of proteins from influenza, including hemagglutinin (HA) and neuraminidase (NA) surface glycoproteins, and nucleoprotein (NP), a highly conserved internal protein have been shown to variably protect against influenza virus infection. Here we demonstrate the utility and efficacy of PIV5-vectored vaccines expressing different influenza antigens. We show that a single immunization with PIV5 expressing the HA from H5N1 highly pathogenic avian influenza (HPAI) induces potent neutralizing antibody responses and protects against a lethal homologous virus challenge. Similarly, immunization with PIV5 expressing the NA antigen from H1N1 or H5N1 influenza induces neuraminidase-inhibiting antibodies and T cell responses, which protect against lethal challenge with homologous and heterosubtypic influenza virus. Finally, we demonstrate that immunization with PIV5 expressing influenza NP induces potent T cell responses and protects against homologous (H5N1) and heterosubtypic (H1N1) influenza virus challenge. Thus, PIV5-vectored vaccines offer diverse and effective means for inducing potent and broadly reactive immune responses to protect against seasonal and pandemic influenza infections. Details on mechanism of action, options for administration, and applications for agricultural and human health are discussed..

281 **Virus-like Particles as Vaccines for Paramyxoviruses**

Pramila Walpita
School of Medicine, University of Hawaii at Manoa

Human use vaccines are available for only two of the many highly pathogenic paramyxoviruses, namely for measles and mumps. For several others, such as RSV, hMPV and hPIV3, and the highly lethal zoonotic Nipah virus (NiV), vaccines are much needed. We have made NiV-like particles (NiV-VLPs) composed of the two surface glycoproteins, and the matrix protein, all three retaining their native properties. We have reported the vaccine potential of these particles by showing that they had many virus-like features such as their dense, ordered and repetitive surface structure; their syncytiogenic property with potential implications for vaccine potency; and their ability to induce neutralizing antibody response. We are now taking this work to the next stage by undertaking protective efficacy studies. In further work, we have produced other NIV-VLP vaccine candidates that include additional viral components such as the N protein, The rationale for these latter compositions is the fact that in some closely related paramyxoviruses like measles virus, these internal components are known to modulate host innate immunity which may impact on type and duration of adaptive immunity and protection conferred by a VLP vaccine. The significance and added value of this research is that the knowledge gained through these studies will refine and direct the development of VLP vaccines for other paramyxoviruses such as RSV. To this end, we have made RSV VLPs and tested them in vitro, and in the cotton rat model of RSV disease for adaptive immunity and protection from challenge.

abstracts

282 **The human respiratory syncytial virus (hRSV) is an ubiquitous pathogen that causes severe lower respiratory tract infection worldwide**

Marie-Anne RAMEIX-WELTI (1, 2), Julien SOURIMANT (1, 3), Jenna FIX (3), Aude REMOT (3), Sabine RIFFAULT (3), Elyanne GAULT (1,2), Jean-Francois ELEOUET (3)
1) Université Versailles St-Quentin, EA 3647 Epidémiologie et Physiopathologie des Infections Microbiennes, Université de Versailles St-Quentin-en-Yvelines –UFR des Sciences de la Santé, 2 av de la Source de la Bièvre 78180 MONTIGNY-LE-BRETONNEUX
(2) AP-HP, Laboratoire de Microbiologie, Hôpital Ambroise Paré, 9 avenue Charles-de-Gaulle 92100 BOULOGNE-BILLANCOURT
(3) INRA Centre de Recherche de Jouy-en-Josas, Unité de Recherche Virologie et Immunologie Moléculaire, Domaine de Vilvert 78352 JOUY-EN-JOSAS cedex

The human respiratory syncytial virus (hRSV) is an ubiquitous pathogen that causes severe lower respiratory tract infection worldwide in young infants as well as in elderly or immunosuppressed adults. The lack of knowledge of its replication mechanisms is a limit for the development of specific antiviral drugs. We have set up a reverse genetic system for hRSV based on coexpression of L, P, N and M2-1 proteins and a complete hRSV antigenome of RSV long strain in BSRT7 cells. Different restriction sites were inserted at intergenic regions allowing easy modification of hRSV genome. We successfully rescued a wild type hRSV recombinant virus and recombinant hRSV expressing mCherry or firefly luciferase. These viruses were shown to be stable and to grow almost as well as the parental virus. Expression of mCherry is correlated to infection rate, allows the monitoring of RSV multiplication in cell culture and can be measured in 96 wells cell culture plate using a fluorimeter. Such in vitro fluorescent assay will be useful to screen antiviral molecules or to study seroneutralization. Luciferase expressing RSV is under characterization but may be even more sensitive for these applications. Mice were challenged with recombinant hRSV and hRSV-mCherry. Pulmonary viral loads, measured by q-RT-PCR, were not significantly different whether mice were infected by recombinant wild type hRSV or hRSV-mCherry. Replication of luciferase virus in mice is under evaluation. These recombinant viruses may be useful to detect infected cells in vivo and to monitor the efficiency of antiviral strategies in mouse model.

283 **An eight segments swine influenza virus harbouring H1 and H3 hemagglutinins as a LAIV candidate for pigs**

Hyun-Mi Pyo 1, Aleksandar Masic3, Shawn Babiuk 2 and Yan Zhou 1*
1 Vaccine and Infectious Disease Organization, University of Saskatchewan, Saskatoon, SK S7N 5E3, Canada
2 National Centre for Foreign Animal Disease-Canadian Food Inspection Agency, Winnipeg, MB, R3E 3M4, Canada
3 Bioniche Life Sciencies Inc, Belleville, ON K8N 5J2, Canada

Swine influenza virus (SIV) infections cause significant economic loss in the pork industry and poses public health concern. Currently used SIV vaccines for pigs are killed and their protection efficiency in the field is limited. Considering a pandemic potential of a novel influenza viruses resulting from gene reassortnat in pigs, the importance of a vaccination is highlighted as the most effective countermeasure. Live attenuated influenza vaccines (LAIV) provide strong, long-lived, cell mediated and humoral immunity against different influenza subtypes without need for perfect antigen matching. Here we report a generation of new potential LAIV, an eight segment SIV harbouring two different SIV hemagglutinins (H1 and H3) by fusing the H3 HA ectodomain with the cytoplasmic tail, transmembrane domain and stalk region of NA of H1N1 SIV. This H1H3 chimeric SIV showed similar kinetics and growth properties to parental wild type virus in vitro when exogenous neuraminidase is provided. However the H1H3 chimeric SIV was highly attenuated in pigs, demonstrating the great potential to serve as LAIV with broad protection.

284 **Analysis of Complete Genome Sequences of Bovine Parainfluenza Virus Type 3 BN-1 and Vaccine Strain BN-CE for Reverse Genetics**

Takashi Ohkura 1, Takehiko Kokuho 2, Misako Konishi 3, Ken-ichiro Kameyama 3, Kaoru Takeuchi 1

1 Department of Infection Biology, Division of Biomedical Science, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan; 2 Biologics Production, Center for Animal Disease Control and Prevention, National Institute of Animal Health, Tsukuba, Ibaraki, Japan; 3 Viral Disease and Epidemiology Research Division, National Institute of Animal Health, Tsukuba, Ibaraki, Japan 3

In recent years, advanced in reverse genetics technology have enable a number of negative-strand RNA viruses to be genetically modified so that they can utilize as vaccine vectors expressing foreign antigens. Bovine parainfluenza virus type 3 (BPIV3) is enveloped, nonsegmented negative-strand RNA virus of the genus *Respirovirus* in the family *Paramyxoviridae*. The viral RNA genome of BPIV3 is an approximately 15 kbp in length and encodes N, P, M, F, HN and L proteins. The BPIV3 strain, BN-1, isolated from cattle in Japan and attenuated by serial cell culture passages in chicken embryo fibroblast, resulting in the BN-CE strain. The attenuated BN-CE strain has been used as safety live-vaccine for cattle in Japan. To develop a novel recombinant BPIV3 by reverse genetics, which stably expresses various antigens derived from the pathogens of cattle (e.g. envelope proteins of bovine viral diarrhea virus), we initially determined the complete genome sequence of the BN-1 and BN-CE strains. The complete genome of the BN-1 and BN-CE were each 15,480 bp. Phylogenetic analysis classified both strains into genotype A. When the complete genome sequence of the BN-CE strain was compared with that of the parental BN-1 strain, six nucleotide mutations were detected in the BN-CE genome and amino acid changes were also identified, including a leucine-to-isoleucine change at position 287 in F gene and a cysteine-to-serine change at position 387 in L gene. Based on the complete genome sequences of BPIV3, our current study works on the recovery of an infectious BPIV3 from cDNA.

abstracts

285 **2'-Fluoro-2'-deoxypurineriboside ProTides: a step forward towards developing influenza virus polymerase inhibitors**

E. Vanderlinden 1, S. Meneghesso 2, A. Brancale 2, J. Balzarini 1, C. McGuigan 2, L. Naesens 1

1 Rega Institute for Medical Research, KU Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium; 2 Welsh School of Pharmacy, Cardiff University, King Edward VII Avenue, Cardiff CF10 3NB, UK

The potential use of nucleoside analogues as inhibitors of influenza virus is hardly explored. We here determined the inhibitory effect of various base- or ribose-modified GTP derivatives on influenza virus polymerase, using an RNA elongation assay with virion-derived viral ribonucleoproteins (vRNP). The most potent inhibitors of [8-3H]-GTP incorporation were 7-deaza-GTP (IC50: 4.1 μM) and 2'-fluoro-2'-deoxy-GTP (IC50: 3.7 μM). 7-Deaza-GTP proved to be an efficient alternative substrate, while 2'-fluoro-2'-deoxy-GTP acted as chain terminator. This explains the anti-influenza virus activity of 2'-fluoro-2'-deoxyguanosine (FdG) in vitro and in vivo, as reported by others. To improve its intracellular disposition and activation, we applied the double prodrug approach, combining a ProTide motif for direct delivery of the nucleoside 5'-monophosphate and a 6-O-modified guanine to increase lipophilicity and cellular uptake. Several 6-O-Me, 6-O-Et or 6-Cl-modified FdG ProTides displayed activity in a PCR-based virus yield assay (EC99: ~12 μM). Similar data were obtained in the vRNP reconstitution assay, a cell-based viral polymerase inhibition test. The parent nucleoside analogues were inactive at 100-200 μM. Metabolism experiments with carboxypeptidase Y or whole cell lysates showed that the FdG ProTides are readily cleaved to release the 6-O-modified FdG-5'-monophosphate. Efficient removal of the 6-O-substituent on the guanine part was suggested by enzymatic studies with adenosine deaminase, and by molecular docking into a model of ADAL1. Our results set the stage for developing novel ProTide inhibitors of influenza virus, using any of the successful substitutions examined here (i.e. 2'-fluoro; 7-deazaguanine or a 6-O-modified guanine) as a relevant starting point. [Meneghesso, ChemMedChem., 2013]

286 **NICLOSAMIDE AND CURCUMIN ARE POTENT INHIBITORS OF ARENAVIRUS INFECTION**

Antonella Pasquato, Florian Zoppi, Joel Ramos da Palma, Anggakusuma, Eike Steinmann, and Stefan Kunz *1Institute of Microbiology, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland*
2Institute of Experimental Virology, Twincore Centre for Experimental and Clinical Infection Research; a joint venture between the Medical School Hannover (MHH) and the Helmholtz Centre for Infection Research (HZI), Hannover, Germany

Viral hemorrhagic fevers by arenaviruses are devastating emerging human diseases. The lymphocytic choriomeningitis virus (LCMV) is the prototypic member of the arenavirus family. Currently, no FDA approved vaccine or drugs are available against arenaviruses, with the exception of Ribavirin, with limited efficacy. Small molecules with already known mechanisms of action against pathogens are attractive candidates against arenavirus infections. Curcumin is a small compound isolated from the spice turmeric. It has a variety of beneficial properties, including antiinflammatory and chemotherapeutic activities. Curcumin is also known as anti-viral via different mechanisms of action. Other small organic compounds extracted from plants (e.g. green tea catechins) have antiviral properties. Niclosamide is a FDA approved drug against helminthes with antiviral activity due to its ability to deplete endosomal proton gradients. Here, we show that curcumin and niclosamide are potent inhibitors of LCMV. Curcumin shows virucidal activity against LCMV with micromolar range IC50. It blocks LCMV primary infections and reduces virus cell-to-cell propagation. Similarly, niclosamide potently blocks LCMV infection by interference with the entry step of the virus life cycle. The mechanism of action relies on the ability to negatively tune the pH of late endosomes where LCMV fusion takes place. Low cost drugs such as curcumin and niclosamide that can be delivered orally and stored at room temperature in tropical climates may represent promising antiarenavirus candidates.

287 **Generation and evaluation of recombinant, live-attenuated Edmonston-Zagreb measles viruses which express EGFP**

Linda J. Rennick1, Rory D. de Vries2, Thomas J. Carsillo1, Ken Lemon3, Geert van Amerongen 2, Selma Yüksel 2, R. Joyce Verburgh 2, W. Paul Duprex1 and Rik L. de Swart2

1Department of Microbiology, Boston University School of Medicine, Boston, MA 02118, 2Department Viroscience, Erasmus MC, Rotterdam, Netherlands and 3School of Medicine, Dentistry and Biomedical Sciences, Queen's University of Belfast, Belfast, United Kingdom

We generated a reverse genetics system for the live-attenuated measles virus (MV) strain Edmonston-Zagreb (EZ) allowing recovery of recombinant (r)MVEZ. MVEZ is widely used as a vaccine and is currently being evaluated by WHO for aerosol measles vaccination. Introduction of the open reading frame encoding EGFP within an additional transcriptional unit (ATU) at the promoter-proximal position of rMVEZ, or between the phospho- and matrix protein encoding genes allowed the generation of rMVEZEGFP(1) and rMVEZEGFP(3), respectively. Intratracheal inoculation of macaques (n=3 per virus) with 104 TCID50 showed that serum antibody responses in animals vaccinated with rMVEZEGFP(1) were lower and delayed compared to those in animals vaccinated with rMVEZEGFP(3). This led to the question of whether rMVEZEGFP(3) was the optimal virus for vaccination studies, or if a virus expressing EGFP from a more promoter-distal position in the genome would be even better. A third virus, rMVEZEGFP(6), containing an identical ATU between the hemagglutinin and large protein encoding genes was generated. Macaques (n=3 for rMVEZEGFP(3) and n=6 rMVEZEGFP(6)) were infected with 104 TCID50 of each virus by intratracheal inoculation. MV was isolated from broncho-alveolar lavage samples from all animals inoculated with rMVEZEGFP(3), but only four of the animals inoculated with rMVEZEGFP(6). Although all animals seroconverted by day 24 post-vaccination, three of the animals inoculated with rMVEZEGFP(6) did not develop neutralizing antibodies. All animals inoculated with rMVEZEGFP(3) developed neutralizing antibodies and serum responses were comparable to animals vaccinated with non recombinant MVEZ indicating that rMVEZEGFP(3) is the optimal virus for vaccination studies.

288 **Evaluation of avian paramyxovirus serotype 2-10 as vaccine vectors for chickens pre-immunized against Newcastle disease virus**

Ryota Tsunekuni, Hirokazu Hikono, Takehiko Saito
National Institute of Animal Health

Newcastle disease virus (NDV), also known as avian paramyxovirus (APMV) serotype 1, is used as a vector for avian influenza (AI) vaccines. However, NDV vector cannot be used for an emergency vaccination against AI in chickens because commercial chickens often possess pre-existing immunity against NDV induced by a routine vaccination. Therefore, a novel vector should be developed for an emergency vaccination against AI in chickens to control severe AI outbreaks. We hypothesize that APMV serotype 2-10 can be candidates for such vectors because it is likely that they are, like NDV, suitable for mass vaccination through spray or drinking water and induce immune responses in mucosal tissues where AI viruses primarily replicate. Here, we evaluated the immunogenicity and replication of APMVs in chickens under pre-existing immunity against NDV. Results showed that, among APMV serotype 2-10, viruses of serotype 2, 6, and 10 were less cross-reactive to antibodies against NDV in hemagglutination inhibition and virus neutralization tests. Virus replication in mucosal tissues as well as antibody response after oculonasal inoculation was observed when two-week-old chicks were challenged with APMVs of serotype 2, 6, and 10. They also replicated in mucosal tissues and induced antibody responses in chickens that were vaccinated twice with NDV prior to the challenge. These results warrants further study to develop vaccine vectors based on APMV serotype 2, 6, 10 for emergency vaccination against AI in chickens.

289

290 **A novel antiviral compound displays potential broad-spectrum activity through a dual mechanism of action.**

Oliver Dibben, Heinrich Hoffmann, Megan L. Shaw
Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York NY 10029, USA

Influenza viruses continue to pose a major public health threat worldwide and options for antiviral therapy are limited by the emergence of drug-resistant virus strains. Additionally, the repertoire of drugs currently licensed for treatment of influenza act through a limited range of mechanisms. Namely: inhibition of the neuraminidase enzyme, by oseltamivir and zanamivir; or blocking of the M2 ion channel, by the adamantanes. Combination therapies, as exemplified by antiretroviral therapy, are likely to become the paradigm for treating a range of viral infections, including influenza. Targeting virus replication via multiple mechanisms will help limit the emergence of resistant viruses and subsequently extend the lifespan of drugs. For this purpose novel compounds with a more diverse range of antiviral mechanisms are needed. A cell-based, single-cycle assay was used to identify potential inhibitors of influenza virus replication. We describe a novel compound, M7, which inhibits the replication of influenza A and B viruses, as well as non-segmented negative sense RNA viruses. Further to its broad-spectrum properties M7 acts on two independent stages of the virus life cycle. In pseudotyped-particle entry assays M7 demonstrates activity as an entry inhibitor, while efficacy in minigenome assays indicates an ability to inhibit viral polymerase activity. This suggests that M7 inhibits the replication of a diverse range of viruses through two mechanisms, most likely by targeting a cellular function involved in both stages. A drug with broad-spectrum properties and a dual mechanism of action could prove invaluable in the combination therapy approach to treating virus infection.

291 **Two new broadly neutralizing human antibodies binding to the highly conserved stem-epitope of Influenza A hemagglutinin**

Wyrzucki A (1), Bianchi M (1), Dreyfus C (2), Blattmann B (3), Steck M (1), Kohler I (1), Grütter M (3), Wilson I (2), Hangartner L (1)
(1) Institute of Medical Virology, University of Zurich, Switzerland
(2) The Scripps Research Institute, La Jolla, CA, USA
(3) Department of Biochemistry, University of Zurich, Switzerland

Influenza A viruses can rapidly mutate their antigenic structures as strategy to escape pre-existing immunity. Vaccines to influenza therefore only protect against the immunizing or closely related strains, and have to be annually reformulated. A universal vaccine that induces broad immunity against multiple subtypes of influenza A viruses, including newly emerging strains, is a long-sought goal in medical research. To date, different human monoclonal heterosubtypic antibodies (mhAb) recognizing more than one hemagglutinin (HA) subtype have been isolated. Crystal structures revealed that these mhAbs mainly interact with the stem region of HA. Most mhAbs only neutralized either group1 or group 2 viruses. However, two clones with neutralizing activity against viruses from both phylogenetic groups have been recently described. To extend the knowledge about universal HA epitopes, a phage display Fab library was prepared from the immune repertoire of a single donor. Using a proprietary selection procedure, we obtained a set of Fab clones displaying distinct HA cross-reactivity patterns. The two most promising candidates neutralized viruses from group 1 (mhAb 3.1) or both group 1 and group 2 (mhAb 1.12) subtypes, with mhAb 1.12 being broad neutralizing all tested subtypes (H1 to H15). Crystal structure, kinetic properties and mode of action were analyzed for both clones, defining the recognized epitope as the highly conserved HA2 stem region. At present, a novel generation of immunogens capable of eliciting protective heterosubtypic antibodies to a broad spectrum of Influenza A viruses is in development
This work was financed by Swiss National Science Foundation grant PP00P3_123429

292 **Antiviral susceptibilities of A(H3N2)v, A(H1N1)v, A(H1N2)v influenza viruses isolated from humans in the United States in 2011-2**

Katrina Sleeman, Vasiliy P. Mishin, Zhu Guo, Rebecca J. Garten, Amanda Balish, Julie Villanueva, James Stevens, and Larisa V. Gubareva *Virus Surveillance and Diagnosis Branch, Influenza Division, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA*

Since 2011, outbreaks caused by variant influenza A viruses with evidence of limited human-to-human transmission have become a public health concern. Except for a single A(H1N2)v virus, all variant viruses tested shared the A(H1N1)pdm09 M gene containing the marker of M2 blocker resistance, S31N. To assess susceptibilities to FDA-approved (oseltamivir and zanamivir) and other (peramivir, laninamivir, and A-315675) neuraminidase (NA) inhibitors (NAIs), viruses were tested in the fluorescent NA inhibition (NI) assay. All recovered A(H3N2)v viruses (n=156), with the exception of a single virus, A/Ohio/88/2012, isolated from an untreated patient, as well as all A(H1N2)v and A(H1N1)v viruses were susceptible to the NAIs tested. The A/Ohio/88/2012 A(H3N2)v virus showed 35-, 65-, and 7-fold reduced inhibition by oseltamivir, zanamivir, and laninamivir, respectively, when compared to the other A(H3N2)v viruses. Two rare substitutions were identified in the NA of A/Ohio/88/2012, S245N and S247P. Recombinant NA proteins containing these substitutions, alone or in combination, were tested. The presence of S247P alone and in combination with S245N was accompanied by ~60% reduction in NA activity and elevated IC50s to oseltamivir, zanamivir, and laninamivir. Using cell culture based assays the antiviral agents, favipiravir (T-705) and DAS181, were shown to inhibit replication of variant influenza viruses, including A/Ohio/88/2012. The findings reported here demonstrate the necessity of continued surveillance for the identification of newly emergent influenza variants and the monitoring of their susceptibility to antiviral medications.

293 **Rapid strategy for screening by pyrosequencing of influenza reassortants for**

Tatiana Bousse, Svetlana Shcherbik, Marnie Levine and Alexander Klimov
Center for Disease Control and Prevention Rapid strategy for screening by pyrosequencing of influenza reassortants for generation of live attenuated
Vaccines Virus Surveillance and Diagnosis Branch, Influenza Division, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention.

Live attenuated influenza vaccine viruses (LAIVs) can be generated by classical reassortment of gene segments between a cold- adapted (ca), temperature- sensitive (ts) and attenuated (att) master donor virus (MDV) and a seasonal wild type (wt) virus. The vaccine candidates contain six viral genes from the MDV strain, and hemagglutinin (HA) and neuraminidase (NA) genes derived from the circulating wt virus (6:2 genomic composition). Fast and effective selection of viruses with the 6:2 genomic compositions from the large number of genetic variations generated during reassortment is essential for the annual production schedule of vaccine seed viruses. This study describes a new pyrosequencing-based approach for genotypic analysys LAIV reassortant clones. We created LAIV candidate viruses A/Ohio/02/2012(H3N2)-CDC-LV3A and B/Texas /06/2011-CDC-LV2B by classical reassortment of A/Ohio/02/2012 (H3N2) with MDV A/Leningrad/134/17/57 (H2N2), and B/Texas/06/2011(Yamagata lineage) with MDV B/USSR/60/69. Using strain-specific pyrosequencing assays designed for viral genes, we were able to detect a mixed virus genotypes in the allantoic fluid during the cloning process, which allowed semi-quantitative assessment of the relative abundances of segment variants in mixed populations. This approach was used in selecting specific clones for subsequent cloning procedures. The present study demonstrates that pyrosequencing analysis is a useful technique for rapid and reliable genotyping of reassortants and intermediate clones during the preparation of LAIV candidates, and can expedite the generation of vaccines.

294 **A 900,000 small molecule screen reveals potent anti-influenza and broad-spectrum antiviral compounds.**

Kris M. White,1 Paul De Jesus,2 Pablo Abreu,1 Quy Nguyen,2 Atsushi Inoue,2 Silke Stertz,1 Renate Koenig,2 Adolfo Garcia-Sastre,1 Peter Palese,1 Sumit K. Chanda,2 and Megan L. Shaw1
1 Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA.
2 Infectious and Inflammatory Disease Center, Sanford-Burnham Medical Research Institute, 10901 North Torrey Pines Road, La Jolla, CA 92037, USA.

Increasing resistance of seasonal and pandemic influenza A viruses to current antiviral drugs licensed for clinical use has emphasized the need for development of new anti-influenza compounds. Therefore, an ultra-high throughput screen was performed to identify novel small molecule inhibitors of influenza virus replication. The screen assay employed a recombinant influenza A/WSN/33 virus containing the Renilla luciferase gene in place of the influenza HA gene and utilized a MDCK cell line stably expressing the influenza HA protein to support multi-cycle viral replication. The screen yielded a hit-rate of 0.5%, of which the vast majority show little cytotoxicity at the inhibitory concentration. The top 5 compounds from this screen have demonstrated impressive anti-influenza activity, each having an IC50 in the nanomolar range. Further, two of our top leads appear to have broad-spectrum potential with each inhibiting disparate viruses such as those with negative-strand RNA or double-stranded DNA genomes. Finally, we have begun to elucidate the mechanism of action of the top 5 compounds, with two of the influenza-specific compounds inhibiting the function of the influenza virus NP protein via differing mechanisms and another inhibiting HA-mediated membrane fusion. Of the broad-spectrum leads, we have found one to inhibit the de novo pyrimidine synthesis pathway while the other clearly acts through another mechanism and presents a very interesting pattern of viral specificity. This high-throughput screen has yielded many promising new lead compounds, which we hope will shed light on the molecular mechanisms of viral infection and potentially be developed for future clinical use.

295 **Antibody-mediated inhibition of Marburg virus budding**

Masahiro Kajihara,1 Andrea Marzi,2 Eri Nakayama,1 Takeshi Noda,3 Manabu Igarashi,4 Makoto Kuroda,1 Rashid Manzoor,1 Keita Matsuno,1 Heinz Feldmann,2 Reiko Yoshida,1 Yoshihiro Kawaoka,3,5,6,7 and Ayato Takada,1,8
1 Division of Global Epidemiology, Hokkaido University Research Center for Zoonosis Control 2 Laboratory of Virology, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rocky Mountain Laboratories 3 Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo
4 Division of Bioinformatics, Hokkaido University Research Center for Zoonosis Control 5 International Research Center for Infectious Diseases, Institute of Medical Science, University of Tokyo 6 ERATO Infection-Induced Host Responses Project 7 Department of Pathobiological Science, University of Wisconsin—Madison, Madison 8 School of Veterinary Medicine, the University of Zambia

The envelope glycoprotein (GP) of Marburg virus is responsible for virus entry into host cells and known as the only target of neutralizing antibodies. In general, it is believed that neutralizing antibodies inhibit GP-mediated entry of MARV into host cells. Here, we report that MARV GP-specific monoclonal antibodies AGP127-8 and MGP72-17, which do not show neutralizing activity, drastically reduced the budding and release of progeny viruses from infected cells. These antibodies similarly inhibited the formation of virus-like particles (VLPs), whereas the Fab fragment of AGP127-8 showed no inhibitory effect. Morphological analyses revealed that filamentous VLPs were bunched on the surface of VLP-producing cells cultured in the presence of the antibodies. In order to identify the antibody epitopes, we selected escape mutants of chimeric vesicular stomatitis virus expressing MARV GP by using AGP127-8 and MGP72-17. Sequence analyses determining mutations introduced in variant GPs indicated that the epitopes of AGP127-8 and MGP72-17 were located in amino acid positions 411-430, near the furin cleavage site at the C-terminus of GP1 subunit of MARV GP. The present study demonstrates a novel mechanism of the antibody-mediated inhibition of MARV infection, in which antibodies arrest unreleased virus particles on the cell surface. Our data lead to the idea that budding inhibition antibodies contribute to protective immunity against MARV and that the “classical” neutralizing activity is not the only indicator of a protective antibody that may be available for prophylactic and therapeutic use.

296 **In Vitro and In Vivo Evaluation of DHODH as a Host Target for Antiviral Drug Development**

Nagraj Mani, Joshua Leeman, Raj Kalkeri, Steven Jones, Colleen McNeil, Carlos Faerman, Ioana Davies, Alice Tsai, Mark Ledeboer, Michael Clark, Chao Lin, Rene Rijnbrand, Randy Byrn
Vertex Pharmaceuticals, 130 Waverly Street, Cambridge, MA 02139, USA

Several phenotypic antiviral screens were performed to identify chemical starting points for drug discovery targeting multiple respiratory viruses. Among the hits were a subset of compounds that lost activity when uridine was added to the test media suggesting that pyrimidine biosynthesis pathway was part of their antiviral mechanism. These compounds belonged to the quinoline (QNL) and the tetrahydroindazole (THI) chemical classes with structural similarities to brequinar (BRE) and leflunomide (LEF), two known/marketed inhibitors of dihydroorotate dehydrogenase (DHODH), a key enzyme in this pathway. These compounds showed potent antiviral activity against Flu A and B, RSV and PIV and inhibited DHODH in enzymatic assays. However, they also inhibited cell growth resulting in a narrow therapeutic index. Antiviral activity of BRE, LEF and our lead QNL compound was evaluated in an influenza A mouse model. BRE and LEF were administered at doses previously shown to be active in mouse models of other diseases. None of the three compounds showed any signs of antiviral activity *in vivo*. However, there were signs of toxic effects in mice confirming that the compounds had reached pharmacologically active levels. DHODH inhibitors are readily identified in phenotypic screens. However, approved DHODH inhibitors are not commonly used as antivirals. We evaluated DHODH inhibitors identified in antiviral screens and confirmed their *in vitro* antiviral activity. However, the established immunomodulatory effects of DHODH inhibitors, their poor cytotoxicity profile, their loss of antiviral efficacy in presence of biologically-relevant uridine levels and lack of *in vivo* activity indicates that their development as potential antiviral drugs is limited.

297 **In ovo administration of chimeric recombinant Newcastle disease virus expressing VP2 protein of infectious bursal disease virus**

Jinying Ge, Xijun Wang, Meijie Tian, Zhiyuan Wen, Qiulin Feng, Honglei Gao, Xiaole Qi, Xiaomei Wang, and Zhigao Bu
Key Laboratory of Veterinary Public Health of Ministry of Agriculture, State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin 150001, China

The protection of poultry from the highly virulent infectious bursal disease virus (IBDV) and Newcastle disease virus (NDV) can be achieved through vaccination. We generated a chimeric virus with the L gene of Clone-30 and a recombinant chimeric virus expressing the VP2 protein of IBDV. The safety and efficacy of the recombinant chimeric viruses were evaluated. No significant changes in the hatchability and global survival rate were observed after vaccination with the rLaC30L-VP2 vaccines at doses of 104.5 mean embryo infectious doses (EID50) compared to phosphate-buffered saline (PBS)-inoculated embryos. The rLaC30L-VP2 construct induced the production of anti-IBDV and anti-NDV in specific pathogen-free (SPF) birds at one week post hatch. At 22 days post hatch, administration of rLaC30L-VP2 provided 100% protection against highly stringent lethal challenge by NDV and 83.3% protection against very virulent IBDV (vvIBDV) in SPF chickens, with 100% protection of broilers. The rLaC30L-VP2 construct was the most safe and effective vaccine against NDV and IBDV among three vaccines in this study. We propose that this vaccine has potential as an in ovo, attenuated, bivalent live vaccine against infectious bursal disease and Newcastle disease viruses.

298

NIAID RESOURCES FOR THE GLOBAL RESEARCH COMMUNITY TO SUPPORT DEVELOPMENT OF NEW VACCINES AND ANTIVIRALS FOR SEVERE VIRAL INFECTI

Amy E. Krafft, Ph.D.
National Institutes of Health (NIH), National Institute of Allergy and Infectious Diseases (NIAID), Bethesda, MD, USA

The U.S. Government supports R&D programs to develop new diagnostics, vaccines, and therapeutics to improve options for the control of severe human and animal infections caused by negative-strand RNA viruses (NSV). NIAID issues solicitations for proposals to fund product development projects for areas of unmet medical needs through grant and contract awards. NIAID has also implemented pre-clinical services (PCS) programs designed to support qualified researchers in academics, non-profits, and industry who are engaged in discovery, early translational development, and evaluation of new vaccine and antiviral candidates. These PCS programs support the development of new approaches and technologies for diagnostics, universal vaccines, and broad-based therapeutics, as well as the traditional one-bug, one-vaccine, one-drug approaches for detection, prevention, and treatment of viral infections. Support services range from proof-of-concept studies in vitro and in animal models of infection to human safety and efficacy testing of the most promising vaccine and therapeutics candidates. These support services are part of a larger NIAID effort to build a comprehensive set of research resources and services to facilitate global efforts to develop the next generation of diagnostics, vaccines, and therapeutics to control emerging infectious diseases and biodefense agents. Investigators interested in more information should review the information provided at http://www.niaid.nih.gov/labsandresources/resources/dmid/Pages/default.aspx and contact us.

299

Real-time monitoring of RSV viral replication in BALB/c mice through detection of luciferase expression using the IVIS® Spectrum

Joshua DiNapoli, Rachel Groppo, Changhong Zhou, Michael Kishko, Darren Smith, Harry Kleanthous, Mark Parrington, Linong Zhang *Sanofi Pasteur*

The murine model is widely used for efficacy testing of RSV vaccine candidates. In the classical application of this model, RSV titer is assessed by culture of virus from lung tissue at the time points of interest. A key limitation of this approach is the large number of animals required as well as time and labor needed for the associated assays, particularly if the goal is to monitor replication kinetics over time. To address this limitation, we have generated a recombinant RSV expressing firefly luciferase (RSV/luc), which has allowed us to monitor RSV-mediated luciferase expression using the IVIS® Spectrum in vivo imaging system as a surrogate for viral replication without the need for animal sacrifice. Following infection of mice with 106 PFU, RSV/luc demonstrated similar growth kinetics to the wild type virus based on standard culture techniques, with peak replication on days 4 - 5 post-infection and clearance by day 7. Luciferase expression mirrored culturable RSV/luc virus titer in the lung; however, luciferase expression was a more sensitive measure of virus replication. Luciferase expression was detected as early as day 1 post infection and as late as day 7 post-infection, whereas both the wild type virus and RSV/luc were only detectable until day 6 using standard culture methods. Taking into account the wild type-like replication of RSV/luc in mice, the sensitivity of luciferase detection, and the substantial reduction in mouse number and labor, the use of RSV/luc in combination with the IVIS® Spectrum in vivo imager is an attractive alternative to classical methods for RSV vaccine efficacy testing in the murine model.

300 **Protective Efficacy of Neutralizing Monoclonal Antibodies in a Nonhuman Primate Model of Ebola Hemorrhagic Fever**

Reiko Yoshida1, Andrea Marzi2, Yasuhiko Suzuki1, Manabu Igarashi1, Friederike Feldmann3, Douglas Brining4, Heinz Feldmann2, and Ayato Takada1
1Division of Global Epidemiology, Hokkaido University Research Center for Zoonosis Control, Sapporo, Japan; 2Laboratory of Virology, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rocky Mountain Laboratories, Hamilton, MT , USA.; 3Office of Operations Management, 4Rocky Mountain Veterinary Branch, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rocky Mountain Laboratories, Hamilton, MT, USA.

Ebola virus (EBOV) is the causative agent of severe hemorrhagic fever in primates, with human case fatality rates up to 90%. Today, there is neither a licensed vaccine nor a treatment available for Ebola hemorrhagic fever. We previously reported that passive immunization with monoclonal antibodies (MAbs) specific to EBOV glycoprotein protected mice and guinea pigs from a lethal EBOV challenge. In this study, we genetically modified two clones of neutralizing MAbs to create human-mouse chimeric MAbs ch133 and ch226 and evaluated their protective potential in a rhesus macaque model of lethal EBOV infection. Reduced viral loads and partial protection were observed in animals given MAbs ch133 and ch226 combined intravenously at 24 hours before and 24 and 72 hours after challenge. MAbs circulated in the blood of a surviving animal until virus-induced IgG responses were detected. In contrast, serum MAb concentrations decreased to undetectable levels at terminal stages of disease in animals that succumbed to infection, indicating substantial consumption of these antibodies due to virus replication. Accordingly, the rapid decrease of serum MAbs was clearly associated with increased viremia in non-survivors. Our results indicate that EBOV neutralizing antibodies may be beneficial in reducing viral loads and prolonging disease progression during Ebola hemorrhagic fever.

301 **A replication-incompetent influenza virus bearing the HN glycoprotein of human parainfluenza virus as a bivalent vaccine**

Hirofumi Kobayashi1, Kiyoko Iwatsuki-Horimoto1, Maki Kiso1, Ryuta Uraki1, Yurie Ichiko1, Toru Takimoto2, Yoshihiro Kawaoka1,3,4,5
1 Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan
2 Department of Microbiology and Immunology, University of Rochester Medical Center; 601 Elmwood Ave., Box 672, Rochester, NY 14642, U.S.A.
3 Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI 53706, USA
4 International Research Center for Infectious Diseases, Institute of Medical Science, the University of Tokyo, Tokyo 108-8639, Japan
5 ERATO Infection-Induced Host Responses Project, Saitama 332-0012, Japan

Influenza virus and human parainfluenza virus (HPIV) are major causes of acute respiratory illness in young children. Inactivated and live attenuated influenza vaccines are approved in several countries, but no vaccine is licensed for HPIV. We previously showed that a replication-incompetent PB2-knockout (PB2-KO) virus that possesses a reporter gene in the coding region of the PB2 segment can serve as a platform for a bivalent vaccine. To develop a bivalent vaccine against influenza and parainfluenza virus, here, we generated a PB2-KO virus possessing the hemagglutinin-neuraminidase (HN) glycoprotein of HPIV type 3 (HPIV3), a major surface antigen of HPIV, in its PB2 segment. We confirmed that this virus replicated only in PB2-expressing cells and expressed HN. We then examined the efficacy of this virus as a bivalent vaccine in a hamster model. High levels of virus-specific IgG antibodies in sera and IgA, IgG, and IgM antibodies in bronchoalveolar lavage fluids against both influenza virus and HPIV3 were detected from hamsters immunized with this virus. The neutralizing capability of the serum antibodies was also confirmed. Moreover, the immunized hamsters were completely protected from virus challenge with influenza virus or HPIV3. These results indicate that PB2-KO virus expressing the HN of HPIV3 has the potential to be a novel bivalent vaccine against influenza and human parainfluenza viruses.

abstracts

302

303 **Mutational analysis of the binding pockets of the diketo acid inhibitor L-742,001 in the influenza virus PA endonuclease**

Annelies Stevaert (1), Roberto Dallocchio (2), Alessandro Dessì (2), Nicolino Pala (3), Dominga Rogolino (4), Mario Sechi (3), Lieve Naesens (1)
(1) Rega Institute for Medical Research, KU Leuven, B-3000 Leuven, Belgium
(2) CNR-Consiglio Nazionale delle Ricerche - Istituto di Chimica Biomolecolare, 07040 Li Punti, Italy
(3) Dipartimento di Chimica e Farmacia, Università di Sassari, 07100 Sassari, Italy
(4) Dipartimento di Chimica, Università di Parma, 43124 Parma, Italy

The influenza virus PA endonuclease, which cleaves capped host pre-mRNAs to initiate synthesis of viral mRNA, is a prime target for antiviral therapy. The diketo acid compound L-742,001 was previously identified as a potent inhibitor of the influenza virus endonuclease reaction, but information on its precise binding mode to PA or its potential resistance profile is limited. Computer-assisted docking of L-742,001into the crystal structure of inhibitor-free N-terminal PA (PA-Nter) indicated a binding orientation distinct from that seen in a recent crystallographic study with L-742,001-bound PA-Nter. A comprehensive mutational analysis was performed to determine which amino acid changes within the catalytic center of PA or its surrounding hydrophobic pockets, alter the antiviral sensitivity to L-742,001 in cell culture. Marked (up to 20-fold) resistance to L-742,001 was observed for the H41A, I120T and G81F/V/T mutant forms of PA. Two- to threefold resistance was seen for the T20A, L42T and V122T mutants, and the R124Q and Y130A mutants were threefold more sensitive to L-742,001. Several mutations situated at non-catalytic sites in PA had no or only marginal impact on the enzymatic functionality of viral ribonucleoprotein complexes reconstituted in cell culture, consistent with the less conserved nature of these PA residues. Our data provide relevant insight into the binding mode of L-742,001 in the PA endonuclease active site. In addition, we predict some potential resistance sites to be taken into account during optimization of PA endonuclease inhibitors towards tight binding in any of the hydrophobic pockets surrounding the catalytic center of the enzyme.

304 **Investigating the functionality and antigenicity of chimeric lyssavirus glycoproteins and their neutralisation profiles**

Jennifer S Evans (1,2), Edward Wright (3), Andrew J Easton (2), Anthony R Fooks (1), Ashley C Banyard (1)
(1) Animal Health and Veterinary Laboratories Agency, Woodham Lane, Weybridge, Surrey, KT15 3NB, United Kingdom
(2) School of Life Sciences, University of Warwick, Coventry, CV4 7AL, United Kingdom
(3) Scool of Life Sciences, University of Westminster, 115 New Cavendish Street, London, W1W 6UW, United Kingdom

Rabies, the archetypal lyssavirus, is one of the most feared viruses known to man and globally, is the cause of more than 50,000 deaths per year. Alongside rabies virus, numerous related lyssaviruses exist that are also capable of causing fatal clinical disease consistent with that seen following infection with rabies virus. Whilst the human burden of these viruses remains unknown, fatalities have been reported. The lyssavirus glycoprotein is the sole target for virus neutralising antibodies and several amino acid epitopes have been linked to virus neutralisation. Lyssaviruses are genetically and antigenically categorised into phylogroups that indicate the level of protection afforded by current vaccines. It is generally accepted that an antibody response to the currently available rabies vaccines affords protection against all viruses that are categorised into phylogroup I. However, this antibody response does not protect against lyssavirus species within phylogroups II and III. Indeed, experimental data has shown that the antibody repertoire induced by rabies virus vaccines is completely unable to neutralise viruses in these phylogroups. In this study we have generated lentivirus pseudotypes containing chimeric lyssavirus glycoproteins that have had their antigenic sites swapped between phylogroup I and II viruses. Using these, we show alteration in both G protein functionality alongside altered neutralisation profiles using a variety of hyperimmune sera. Here we overview results using these chimeric glycoproteins and suggest areas of the G protein responsible for the development of phylogroup specific neutralising responses.

305 **Protection is attributed to both humoral and cellular responses that are reactive against conserved, internal influenza proteins**

Steven F. Baker 1, Hailong Guo 1 4, Randy A. Albrecht 5 6, Adolfo García-Sastre 5-7, David J. Topham 1-4, Luis Martínez-Sobrido 1

Department of Microbiology and Immunology1, New York Influenza Center of Excellence2, Center for Biodefense and Immune Modeling3, David H. Smith Center for Vaccine Biology and Immunology4, University of Rochester, 601 Elmwood Avenue, Rochester, NY 14642; Department of Microbiology5, Global Health and Emerging Pathogens Institute6, Department of Medicine, Division of Infectious Diseases7, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, New York, NY 10029

Constant re-emergence of influenza viruses that escape humoral immune repertoires and that only healthy, 2-49 year olds are recommended for the live-attenuated influenza vaccine demonstrate the need for new vaccines that safely protect against this upper respiratory pathogen. Single-cycle infectious virus vaccines have been shown to be safe and immunogenic vectors in the context of flavivirus, rabies virus, SIV, and HSV. We used plasmid-based reverse genetics techniques to develop a single-cycle infectious Influenza Virus (sciIV) from the pandemic 2009 H1N1 influenza A virus, and here, evaluate its potential as a vaccine. In our sciIV approach, the fourth viral segment, which encodes for the receptor-binding and fusion protein hemagglutinin (HA), has been removed. Upon infection of normal (non-HA expressing) cells, although no infectious progeny are produced, the expression of viral proteins occurs. We found that sciIV vaccination is safe, immunogenic, and protects mice against influenza homologous and heterologous viral challenges. Furthermore, priming with sciIV is dose- and replication-dependent, and protection is attributed to both humoral and cellular responses. Our data indicate that lung-resident CD8 T cells are specific against conserved, internal influenza proteins, and provide a significant impact in limiting challenge virus lung replication. Safety, immunogenicity and protection conferred by sciIV vaccination were also demonstrated in ferrets, where immunization additionally blocked direct and aerosol transmission events. Generating safe vaccines reactive against the internal proteins of influenza that can additionally block transmission of virus are an ideal alternative to current strategies, as they can provide more durable cross-protection against future infection.

306 **Generation of recombinant arenavirus in FDA-approved cell lines: implications for vaccine development**

Benson Y. H. Cheng1, Emilio Ortiz-Riaño1, Juan C. de la Torre2, Luis Martínez-Sobrido1

Department of Microbiology and Immunology1, University of Rochester, 601 Elmwood Avenue, Rochester, NY 14642; Department of Immunology and Microbial Science2, The Scripps Research Institute, La Jolla, California 92037

Arenaviridae is a family of enveloped viruses with a bi-segmented negative strand RNA genome. Several arenaviruses, chiefly Lassa (LASV) and Junín (JUNV) viruses in West Africa and Argentina, respectively, cause hemorrhagic fever (HF) disease in humans that is associated with high morbidity and significant mortality. Notably, increased travel to and from endemic areas has resulted in cases of Lassa fever in non-endemic metropolitan areas. In addition, arenaviruses also pose a biodefense threat. There are no Food and Drug Administration (FDA)-approved arenavirus vaccines and current antiviral therapy is limited to an off-label use of ribavirin. Existing plasmid-based reverse genetic approaches for the generation of recombinant arenavirus have been of great value for virus research, however, they have been limited to the use of murine cells, and therefore non-suitable for human vaccine production. To develop arenavirus reverse genetics in human (293T) and FDA-approved (Vero) cells, we cloned both vRNA segments into plasmids under the control of the human-specific RNA polymerase-I promoter. Using this approach, we have generated recombinant, both bi- and tri-segmented LCMV and the live-attenuated Candid1 strain of JUNV from both 293T and Vero cells. Moreover, we have developed an arenavirus reverse genetics system where the number of plasmids required for the rescue of recombinant arenavirus was reduced in half. These advances in arenavirus reverse genetics have opened new avenues for the development of arenavirus vaccines.

307 **A PB2-KO influenza virus-based bivalent vaccine protects mice against pandemic H1N1 and highly pathogenic H5N1 virus challenge.**

Ryuta Uraki1, Maki Kiso1, Kiyoko Iwatsuki-Horimoto1, Satoshi Fukuyama2, Emi Takashita3, Makoto Ozawa4,5, and Yoshihiro Kawaoka1,2,6,7

1. Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan

2. ERATO Infection-Induced Host Responses Project (JST), Saitama 332-0012, Japan

3. Influenza Virus Research Center, National Institute of Infectious Diseases, Musashimurayama, Tokyo 208-0011, Japan.

4. Laboratory of Animal Hygiene, Joint Faculty of Veterinary Medicine, Kagoshima University, Kagoshima 890-0065, Japan.

5. Transboundary Animal Diseases Center, Joint Faculty of Veterinary Medicine, Kagoshima University, Kagoshima 890-0065, Japan.

6. Department of Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI 53711, USA

7. Department of Special Pathogens, International Research Center for Infectious Diseases, Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo 108-8639, Japan

Vaccination is currently the first line of defense against influenza virus infection. Although inactivated and live-attenuated vaccines are available, each has disadvantages (e.g., immunogenicity and safety). Therefore, novel influenza vaccines that overcome the problems of the currently licensed vaccines are needed. We previously developed a replication-incompetent PB2-knockout (PB2-KO) influenza virus that replicates only in PB2 protein-expressing cells. Here, we generated two PB2-KO viruses whose PB2-coding regions were replaced with the HA genes of either A/California/04/2009 (H1N1pdm09) or A/Vietnam/1203/2004 (H5N1). The resultant viruses induced virus-specific antibodies in the serum, nasal wash, and bronchoalveolar lavage fluid of mice at levels that were comparable to, or in some cases greater than those, induced by a conventional formalin-inactivated vaccine. Furthermore, mice immunized with these PB2-KO viruses were protected from lethal challenges with not only the backbone virus strain, but also strains from which their foreign HAs originated, indicating that PB2-KO viruses with antigenically different HAs could serve as bivalent influenza vaccines. Our replication-incompetent influenza viruses could also be candidate bivalent vaccines for other respiratory infectious diseases.

308 **SHe's a novel target for RSV vaccination**

Bert Schepens (1,2), Sarah De Baets (1,2), Koen Sedeyn (1,2), Pieter Bogaert (1,2), Brian Gilbert (3), Pedro A. Piedra (3), Walter Fiers (1,2) and Xavier Saelens (1,2)
1. Department for Molecular Biomedical Research, VIB, Technologiepark 927, Ghent, Belgium
2. Department of Biomedical Molecular Biology, Ghent University, Technologiepark 927, Ghent, Belgium
3. Baylor College of Medicine Houston, U.S.

RIII)-/- BALB/c mice, indicated that the protection mediated by SHe-specific antibodies strongly depends on Fc Receptors. Moreover, using a conditional cell depletion protocol we could demonstrate that alveolar macrophages play a crucial role in SHe immune serum mediated antiviral activity. Taken together, these data suggest that SHe-specific antibodies reduce RSV replication by macrophage-dependent elimination of RSV infected cells.gRI, FcgDespite decades of research, there is still no vaccine available against Respiratory Syncytial Virus (RSV). We developed a novel RSV vaccine candidate, based on the extracellular domain of the viral Small Hydrophobic protein (SHe). Using a peptide-ELISA we could not detect SHe-specific IgG in reference sera containing varying levels of RSV neutralizing antibodies. We have produced and characterized both genetic and chemical fusions of SHe with a diverse set of antigen-presenting carriers. These carriers included recombinant virus-like particles and pentameric leucine-zippers. Immunization of BALB/c mice and cotton rats with these SHe-based fusion proteins resulted in significant SHe-specific serum IgG titers and reduced lung virus titers following challenge with RSV, as compared to control vaccinated animals. Next to vaccination also passive transfer of SHe-specific antibodies resulted in significant reduction of pulmonary RSV-A replication and associated morbidity. As the SH protein is rare on RSV virions it was not surprising that serum of mice and cotton rats vaccinated with SHe-fusion constructs did not neutralize RSV in vitro. However, in contrast to virions, the surface of RSV infected cells can be efficiently bound by SHe-specific IgG antibodies. Passive immunization experiments using wild type and (Fc

309 **FcgammaRIII Is Dispensable for Protection against Influenza by Matrix Protein 2 Ectodomain-Specific IgG2a**

Silvie Van den Hoecke [1,2], Karim El Bakkouri [1,2], Anouk Smet [1,2], Kenny Roose [1,2], Michael Schotsaert [1,2], Walter Fiers [1,2], Xavier Saelens [1,2]
1: Department for Molecular Biomedical Research, VIB, Ghent, Belgium
2: Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium

The ectodomain of matrix protein 2 (M2e) of influenza A virus is a universal influenza A vaccine candidate. M2e is highly conserved across influenza virus subtypes, and humoral anti-M2e immunity protects against influenza virus challenge in animal models. We recently reported that protection by M2e immune serum requires activating Fcγ Receptors and alveolar macrophages and that an IgG2a-containing serum fraction protected mice in the absence of a functional FcγRIII. In this work, we compared *in vivo* protection by two monoclonal (mAb) antibodies directed against M2e: mAb 37 (IgG1) and mAb 65 (IgG2a). We first demonstrated that these two mAbs recognize the same epitope in M2e, as deduced from an Ala-scan analysis. In addition, Surface Plasmon Resonance measurements showed that the affinity of these two mAbs for the M2e-target antigen is very similar. Passive transfer of mAb 37 or -65 protected BALB/c mice against challenge with an H3N2 virus that was lethal to negative control, isotype-matching mAb recipient animals. MAb 65 also protected mice that were deficient in FcγRIII whereas mAb 37 failed to protect these mice. We also observed significant protection by passive transfer of mAb 65 in (FcγRI, FcγRIII)-/- mice, suggesting that FcγRIV possibly also contributed to protection. We conclude that M2e-specific IgG1 requires FcγRIII for protection in the mouse model. M2e-specific IgG2a isotype antibodies are also protective in the absence of FcγRI or FcγRIII. This finding is important for the clinical development of M2e-based vaccines and may help determine the choice of adjuvant.

310 Hemagglutinin-Neuriminidase from HPIV3 mediates human NK regulation of T cell proliferation via NKp44 and NKp46.

1Samantha McQuaid, 1Sinead Loughran, 1Patrick Power, 2Dermot Walls, 3Maria Grazia Cusi, 4Claes Orvell and 1Patricia Johnson

1Viral Immunology laboratory, School of Nursing and Health Sciences, Dublin City University, Glasnevin, Dublin 9. Ireland.

2Molecular Virology Laboratory, School of Biotechnology, Dublin City University, Glasnevin, Dublin 9,Ireland.

3Department of Molecular Biology, Section of Microbiology, University of Siena, Via Laterina 8, IT -53100 Siena, Italy.

4Division of Clinical Virology, F68, Karolinska University Hospital, SE-141 86 Stockholm, Sweden

HPIV3 is a major respiratory virus in humans, which primarily affects neonates and infants. However, this virus is associated with poor immunological memory, and reinfection throughout life is common. Our group has previous reported the inability of human mixed lymphocytes to respond proliferatively to HPIV3 infected antigen-presenting cells. This is due to NK cell mediated T cell cycle arrest, which was associated with low IL-2 production during infection. In this study, we demonstrate that the HPIV3 surface glycoprotein Hemagglutinin-Neuraminidase (HN) induces this regulatory mechanism during infection. Of additional interest, while HN induces NK regulation of conventional T cell proliferation and T regulatory population is actually enhanced in humans. Furthermore, the already established interaction between HN and the Natural Cytotoxicity receptors NKp44 and NKp46 is responsible for activation of this NK cell mediated T cell cycle arrest. We suggest that this is a plausible mechanism for failed immunological memory in humans which may also explain the successive failures of vaccines that have involved use of this component. We would further suggest that the success of future vaccines against this important pathogen will require the generation of modified HN versions that retain immunogenicity but do not bind/interact with human NKp44 and NKp46. .

311 Antiviral compounds that prematurely activate and disable the paramyxovirus fusion Protein

Ilaria De Vito (1), Laura Palermo (1), Matteo Porotto (1) and Anne Moscona (1,2)

Departments of (1) Pediatrics and of (2) Microbiology and Immunology, Weill Medical College of Cornell University, New York, NY 10021.

The human parainfluenza viruses (HPIVs) enter host cells by fusion of the viral and target cell membranes. This fusion results from the concerned action of the two viral envelope glycoproteins, the receptor binding protein (hemagglutinin-neuraminidase; HN) and the fusion protein (F). The first step is recognition and binding of cell surface sialic acid receptors by HN. HN-receptor interaction leads to activation of the F protein, via a series of conformational changes in F that render it fusion-competent. To promote fusion successfully, the triggering of F must occur when HN is in contact with its receptor and the F protein is proximal to the target cell membrane. We have shown that this timing of activation represents a potential target for intervention, suggesting a new antiviral strategy. The small molecule N-[4-hydroxy-3-(1H-1,2,4-triazol-3-ylmethyl)phenyl] thiophene-2-sulfonamide (CSC11) is an HPIV3 receptor mimic. Interaction of CSC11 with HN results in F-activation prior to receptor binding; the fusion machinery is prematurely activated and disabled, preventing fusion of the viral membrane with target cells and precluding viral entry. While CSC11 provides a proof of concept for this new antiviral strategy, in itself it is effective only at mM concentrations, making it unfeasible in vivo. Here we discuss the identification of more effective compounds based on modifications to CSC11. Preliminary data suggest that these compounds are active in the uM range and merit in vivo investigation.

312 **Development of a live attenuated peste des petits ruminants DIVA vaccine using reverse genetics techniques**

Murali Muniraju1*, Ashley Banyard2, Hubert Buczkowski2 , Carrie Batten1 and Satya Parida1
1 The Pirbright Institute, Ash Road, Pirbright, Surrey,GU24 0NF, UK.
2 Animal Health and Veterinary Laboratories Agency, Woodham Lane, Weybridge, Surrey, KT15 3NB, UK.

Across the developing world peste des petits ruminants virus (PPRV) places a huge disease burden on agriculture, primarily affecting small ruminant production. PPRV is a non-segmented negative strand RNA virus and alongside rinderpest, canine distemper and measles virus belongs to the family *Paramyxoviridae*, genus *Morbillivirus*. The disease is mainly controlled by vaccinating sheep and goats with live attenuated vaccines that provide lifelong immunity. However, as experienced during the global rinderpest eradication campaign, the current PPR vaccines and companion serological tests do not enable serological *d*ifferentiation between naturally *i*nfected and *v*accinated *a*nimals- the so-called DIVA concept. This feature of current vaccines precludes meaningful assessment of vaccine coverage and epidemiological surveillance based on serology in areas where the virus is circulating, which in turn reduces the efficiency of control programmes. By manipulating the genome of PPRV using reverse genetics we have developed both a positively and negatively marked PPRV vaccine. To positively tag the virus we have added eGFP as a novel transcription unit. The current recommended ELISA test for PPRV depends on competition between a PPR specific monoclonal antibody (C77) and antibodies in the test sera. To negatively tag the virus we have mapped the region of the viral H protein that is critical for C77 binding and have mutated residues to generate an H protein that cannot be detected using C77 by immunofluorescence. These two approaches to vaccine development will enable the development of a novel recombinant vaccine that is able to fulfil the DIVA concept.

313 **Coordinated viral replication and TLR-3 activation enhances T cell immunity generated by live attenuated influenza vaccines**

(1)Vicente Pérez-Giron, (2) Alan Belicha-Villanueva, (1)Sergio Gomez-Medina, (1) Jazmina Gonzalez-Cruz, (1)Anja Lüdtke, (2,3)Adolfo García-Sastre and (1)César Munoz-Fontela.
1- 1Heinrich-Pette-Institut, Leibniz Institute For Experimental Virology. Martinistr. 52, 20251 Hamburg, Germany.
2-ICAHN School of Medicine at Mount Sinai, One Gustave L. Levy Place, Box 1124, New York, New York 10029, USA.
3-Global Health and Emerging Pathogens Institute, Mount Sinai School of Medicine.

Mucosal administration of live-attenuated influenza vaccines (LAIVs) have the potential to generate memory T cells directed against broadly conserved influenza epitopes. Vaccine adjuvants are commonly used to enhance the inflammatory response against inert or poorly immunogenic epitopes. However, the use of adjuvants to enhance or polarize the immune response induced by LAIVs remains unexplored. In this study we have investigated the effect of polyI:C (pIC) a toll-like receptor (TLR)-3 ligand on the maintenance of CD8+ memory T cells in the context of mucosal influenza vaccination. Consistent with its ability to induce type I interferons (IFNs), when administered intranasally together with LAIV, pIC induced an antiviral state and abolished vaccine replication resulting in loss of vaccine protection. However, administration of pIC post-vaccination allowed proper vaccine replication and increased the migration of antigen-loaded lung dendritic cells (DCs) to the lung-draining lymph nodes in a TLR3-dependent manner. This optimal arrival of antigen-presenting cells enhanced the generation of central memory T cells (TCM) that improved viral clearance and survival after lethal influenza challenge. Our results indicate that timely administration of TLR3 ligands may serve as a therapeutic strategy to enhance T cell immunity induced by LAIVs. Our strategy may contribute to provide means not only to reduce the amount of vaccine stock necessary to protect individuals at risk but also to guarantee higher prophylactic perspectives to face future influenza pandemics.

314 **Investigating mechanisms of influenza polymerase host adaptation**

Anna Cauldwell, Jason Long, Hongbo Zhou, Olivier Moncorge, and Wendy Barclay.
Imperial College London

Typical avian influenza A viruses do not replicate efficiently in mammals. Many host adaptive mutations map to the polymerase complex (PB1, PB2 and PA) however the mechanism/s of host adaptation are unknown. Polymerase activity is often measured using a cell-based assay in which the active enzyme is reconstituted from its constituent parts expressed from plasmids. Using this approach we suggest that different PB2 humanising mutations do not enhance polymerase activity by a universal mechanism and furthermore are not all host specific as some enhance activity in avian as well as mammalian cells. We wished to address why certain mutations which enhanced activity in the reconstituted polymerase assay are not selected for in nature and to explore whether the polymerase assay truly reflects viral fitness. To investigate this we used reverse genetics to create a series of viral variants carrying mutations in the PB2 gene that enhanced polymerase activity in the in vitro assay. We carried out virological assays as well as measures of transcription and replication in the context of replicating virus and also analysed the effects of the mutations in vivo. Some mutations that increased in vitro polymerase activity lead to attenuated virus replication that resulted from an increase in interferon activation. One traditional avian influenza virus did not tolerate the famous E627K mammalian-adapting change in PB2 due to RNA incompatibility with other segments in the virus genetic constellation. Taken together these data help risk assess the likelihood of different avian influenza viruses crossing the host range barrier.

315 **Evolution of the hemagglutinin of pandemic H1N1 (2009): maintaining optimal receptor binding by compensatory substitutions**

Erik de Vries[1], Robert P. de Vries[1,2], Carles Martínez-Romero[3], Ryan McBride[2], Peter J.M. Rottier[1], Adolfo García-Sastre [3], James C. Paulson[2], Cornelis A.M. de Haan[1]
1. Virology Division, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands.
2. Department of Chemical Physiology, The Scripps Research Institute, San Diego, USA.
3. Department of Microbiology and Department of Medicine, Division of Infectious Diseases, Icahn School of Medicine Mount Sinai, New York, NY, USA

A pandemic influenza A virus (pdmH1N1) was introduced in April 2009. In three years it has spread in large parts of the population and genetic changes that counteract adaptive immunity are expected to occur. However no major antigenic differences have been reported yet. Recent publications demonstrated that changes in virus binding avidity could provide the first step in counteracting immune-pressure and are likely to precede antigenic changes. We therefore examined the effect of the most frequently occurring amino substitutions in the hemagglutinin (HA) of pdmH1N1 (in NCBI flu database before 1-10-2012) on the receptor-binding properties of recombinant soluble HA trimers. Only two changes (S186P and S188T) increased binding avidity whereas two other substitutions (A137T and A200T) decreased binding avidity (hemaglutination and solid phase ELISA). Construction and analysis of an HA protein tree reveals the worldwide emergence of several HA variants in the latest seasons. The consecutive substitutions that formed these variants were reconstructed stepwise in the recombinant soluble HA trimers. The two major variants both harbored combinations of substitutions (S186P/A137T and S188T/A200T respectively) with opposite individual effects on binding. Strikingly, the combination of these substitutions restores binding avidity and binding specificity (glycan array analysis) to the same level and specificity as observed for the original virus. The results strongly suggest that the HA of pdmH1N1 was already optimally adapted to the human host upon its emergence in april 2009. Moreover, as predicted, transient increases in binding avidity might be one of the first adaptations involved in counteracting antibody neutralization.

316 **HEMAGGLUTININ (HA) ACID STABILITY REGULATES H5N1 INFLUENZA VIRUS REPLICATION, VIRULENCE, TRANSMISSION & INTERSPECIES ADAPTATION**

Hassan Zaraket, Rebecca M. DuBois, Olga Bridges, Sun-Woo Yoon, Richard J. Webby, Stephen W. White, Robert G. Webster, and Charles J. Russell
St. Jude Children's Research Hospital, Department of Infectious Diseases, Memphis, TN 38105, U.S.A

The prefusion form of the HA surface glycoprotein is trapped in a metastable conformation and triggered by low pH to undergo irreversible structural changes that cause membrane fusion in the endosome. Our surveillance studies reveal that HA proteins from human seasonal influenza viruses are activated at pH 5.1-5.4 while those from highly pathogenic avian H5N1 influenza viruses are activated at pH 5.5-6.0. Using sequence analysis, x-ray crystallography, and biochemistry, we have identified numerous mutations that alter the HA activation pH of H5N1 influenza viruses. Using reverse genetics, we have generated recombinant H5N1 influenza viruses that vary in HA activation pH but not in other properties such as HA protein expression, cleavage, or receptor binding. We have found that the optimal HA activation pH for high H5N1 virus growth, virulence, and transmission in avian species (including chickens and ducks) is ~5.6-6.0. An HA2-K58I mutation that decreases the H5N1 HA activation pH from 5.9 to 5.4 was found (a) to attenuate virus growth and eliminate virulence and transmission in ducks, and (b) enhance virus growth in the upper respiratory tracts of mice and ferrets. Overall, HA acid stability is identified as a novel molecular marker for H5N1 adaptation to mammals and constitutes an important risk factor for pandemic potential. Just as changes in the activation energy of the HA protein influence the pathogenicity and interspecies adaptation of emerging H5N1 influenza viruses, other enveloped viruses may also regulate their biological properties by introducing stabilizing or destabilizing mutations in their fusion glycoproteins.

317 **Evolution-guided analysis of primate MxA proteins: the flexible loop L4 is a major determinant for its antiviral specificity**

Corinna Patzina (1), Patrick S. Mitchell (2), Michael Emerman (2), Otto Haller (1), Harmit S. Malik (2), Georg Kochs (1)
(1) Institute for Virology, University of Freiburg, Germany,
(2) Fred Hutchinson Cancer Research Center, Seattle, USA

Human MxA is an interferon-induced antiviral effector protein that has an exceptionally broad spectrum of antiviral activity against diverging families of RNA viruses (e.g. orthomyxo- or bunyaviruses). MxA specifically recognizes viral ribonucleoproteins (vRNPs) of influenza virus, but it is still unclear how inhibition of virus replication is mediated and how such a diverse set of viruses can be blocked. By an evolution-guided analysis of human and non-human primate MxA sequences, we identified a limited set of amino acids under strong positive selection, an indication for host-pathogen interfaces. MxA consists of an N-terminal globular G domain, a bundle signalling element and a C-terminal stalk domain with effector functions, from which an unstructured loop L4 protrudes that contains most of the fast evolving residues. By detailed analysis of this flexible and surface-exposed loop L4, we were able to characterize the minimal sequence required for vRNP recognition and antiviral action against orthomyxoviruses. However, these sites are not critical for the inhibition of bunyavirus replication, indicating that diverse structural elements in loop L4 are responsible for the recognition of different viral components. We thus hypothesize that loop L4 is a flexible and modular structure that evolved as recognition interface for a broad range of viral pathogens.

318 **Modulating paramyxovirus polymerase function rationally and randomly: towards tunable attenuation**

Linda J. Rennick and W. Paul Duprex *
Department of Microbiology, Boston University School of Medicine, Boston, MA 02118, USA

Normally epitope tags and enhanced green fluorescent protein (EGFP) are fused to the amino or the carboxyl terminus of a protein to aid purification and/or visualization in vitro and in vivo. Bioinformatics can help to identify internal, flexible hinges which oftentimes separate conserved regions (CR) in proteins and these can be probed for their ability to be lengthened or modified using well-characterized epitope tags. We have previously used this approach to perform single step attenuation of morbilliviruses by targeting a variable hinge in the large (L) protein. This hinge is located prior to CRVI which functions as a methyltransferase. We have developed wild-type reverse genetics systems for a number of paramyxoviruses and have shown that these bioinformatics-based approaches are transferable to measles, mumps and human respiratory syncytial viruses. From these studies it is clear that a bioinformatics-based approach can only take us so far as insertion of foreign sequences often completely abrogates polymerase function. To overcome this we developed a random insertional mutagenesis library-based approach to identify novel regions of the polymerase which can tolerate insertions. Polymerase function was assessed using bicistronic minigenome replication/transcription assays and a panel of L proteins with differential activities was identified. Tagging the L protein with EGFP permits us to detect the polymerase in living cells and we have used a disease-relevant primary normal human bronchial epithelial cell model to study the polymerase location. Modulating polymerase function rationally to down tune pathogenesis may allow the development of vaccines which are optimally attenuated and safe.

319 **Evidence for Henipavirus spillover into human populations in Africa.**

Olivier Pernet 1, Shannon M Beaty 1, Matthew Lebreton 2, Brad Schneider 2, Nathan Wolfe 3, Benhur Lee 1
1 UCLA - David Geffen School of Medecine/MIMG Dept.
2 Metabiota
3 Global Viral

Zoonotic transmission of henipaviruses (HNV) from their natural fruit bat reservoirs to humans can result in mortality rates in excess of 90%. A recent global study discovered at least 23 distinct viral clades within the henipavirus genus; the known Nipah and Hendra viruses in SE Asia and Australia represents only two of those clades with greatest phylogenetic diversity of henipaviruses present in Africa. To determine the potential for Henipavirus outbreaks or spillovers among humans in Africa, we examined well-curated sets of bat (Eidolon helvum) and human serum samples from southern Cameroon using a highly sensitive (98%) and specific (100%) VSV-based pseudotype seroneutralization assay, and detected NiV-Env cross-neutralizing antibodies in 55% (25/45) and 3% (7/227) of the bat and human sera, respectively. Human sera samples were from individuals at high risk for zoonotic exposure. A detailed analysis of 21 parameters showed that butchering bush (bat) meat was the highest risk factor associated with HNV-seropositivity. Moreover, most of these seropositive samples were collected from the same area – 75% of them in the same village – between 2001 and 2003. Interestingly, this area contains environmental features known to be involved in previous HNV outbreaks in SE Asia, and could be considered a hot spot for viral emergence as it was for the origin of the recently characterized PTLV. In the various taxonomic schemes proposed for the transitional dynamics of zoonotic pathogens, all these features justifiably place henipavirus at or close to the penultimate stage for sustained transmission in human outbreaks.

320 **High potential of canine distemper virus in the ability to use macaca and human Receptors**

Kouji Sakai1, Fumio Seki1, Maino Tahara1, Noriyuki Otsuki1, Yasushi Ami1, Ryoji Yamaguchi2, Masayuki Saijo1, Katsuhiro Komase1, Shigeru Morikawa1, Makoto Takeda1
1: National Institute of Infectious Diseases, Tokyo, Japan
2: Department of Veterinary Pathology, Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan.

Recently outbreaks of canine distemper virus (CDV) occurred in China. In Japan, a CDV outbreak also occurred in monkeys imported from China. Phylogenic analysis revealed continuing chains of CDV transmission in monkeys. Thus, CDV is now a real threat for monkeys, and may be a potential risk for humans. In this study, the ability of CDV to utilize dog, macaca, and human immune and epithelial cell receptors (SLAM and nectin4, respectively) was analyzed. The standard Vero cells and 6 Vero cell lines expressing human, dog, and macaca SLAM and nectin4 were used. A CDV strain (CYN07-dV) isolated from a moribund monkey and 5 CDV strains isolated from distemper dogs were used. In addition, The human SLAM-adapted CDV CYN07-hV strain was used. Using these materials (1) virus growth assay, (2) plasmid-based cell-to-cell fusion assay, and (3) VSV-pseudotyped virus entry assay were performed.
All CDV strains used macaca receptors (both SLAM and nectin4) as efficiently as dog receptors. All CDV strains used human nectin4 as well as dog and macaca nectin4, while only CYN07-hV used human SLAM. Our data revealed that CYN07-hV acquired the ability to use human SLAM via P541S mutation without compromising its intrinsic ability to use other receptors. Thus, the incompatibility of hSLAM is easily resolvable for CDV to cross the species barrier to humans. In addition, our another study suggested that CDV has the potential to counteract the human interferon system and replicate in human cells. Accordingly, CDV should be seriously considered as a potential threat for humans.

321 **Cell entry pathway of an extinct virus.**

Lindsey R. Robinson, Matthijs Raaben, and Sean P.J. Whelan
Department of Microbiology and Immunobiology, Harvard Medical School, Boston, MA, 02115

Endogenous retroviruses (ERVs) and retroviral elements make up 8% of the human genome. While there are no individual fully replication-competent human ERV (HERV) proviruses, several HERV loci encode intact open reading frames and some functional genes. Those include several envelope glycoproteins that are actively expressed and fusogenically functional, including some that are coopted to play an active role in human development. In humans, the most recently endogenized ERV is HERV-K(HML2), which is present at variable copy number.
Expression of the Env gene specifically is associated with human diseases, including several cancers and viral infections. Using a recombinant vesicular stomatitis virus, we replaced the glycoprotein with the envelope protein of HERVK(HML2). The virus, rVSV-HERVK(HML2) is fully replication-competent and efficiently incorporates HERV-K Env into the virions. Characterization of this virus has revealed that unlike most retroviruses, HERV-K Env utilizes a dynamindependent endocytic entry pathway that requires low-pH. Cell fusion experiments confirm thatHERV-K Env requires acidic pH to mediate membrane fusion. Our data identify a novel entry pathway for an extinct virus - a human endogenous retrovirus - and provide tools for further study of endogenization of the virus and investigation of a potential role of this retroviral envelope in human disease.

322 **Measles virus entry pathway governs efficacy of mantle cell lymphoma radio-virotherapy**

Tanner S. Miest, Marie Frenzke, Roberto Cattaneo
Department of Molecular Medicine, Mayo Clinic, and Virology and Gene Therapy track, Mayo Graduate School, Rochester, Minnesota 55905, USA

Virotherapy is an experimental treatment modality that uses replication-competent viruses to treat cancer patients who failed to respond to other therapies. Measles (MV) is a lymphotropic virus currently used in clinical trials of ovarian cancer, glioma, myeloma, and mesothelioma. Mantle cell lymphoma (MCL) is an aggressive B-cell non-Hodgkin's lymphoma incurable using current first line therapies but radiosensitive. To develop a new MCL therapy we armed a vaccine-identical MV vector with the human thyroidal sodium-iodide symporter (NIS), a protein that concentrates iodide within cells and enables non-invasive imaging and combination radio-virotherapy. We used high-resolution single photon emission computer tomography to document vector spread over two weeks in SCID or nude mice. Image resolution was sufficient to discern NIS-expression in the thyroid from that in the proximal salivary glands, and to define the volume of infected tumors and metastases. Infection seeded homogeneously in these, but spread only in well-perfused areas, peaking around day nine in primary Granta-519 MCL xenografts and earlier in metastases. Radio-virotherapy combining our NIS-expressing vector with systemic 131I resulted in more rapid xenograft regression than either therapy alone. We then asked whether cell entry through the primary MV receptor signaling lymphocytic activation molecule (SLAM), or through the vaccine strain receptor CD46, governs oncolysis. Strikingly, only SLAM-dependent entry produced efficient viral spread, tumor regression, extended survival, and sometimes sustained complete remissions. High-resolution, non-invasive imaging technology developed here can facilitate mechanistic studies of virotherapy with different vectors, and of the crucial but elusive early phases of viral replication in the host.

323 **New World bats are reservoirs of diverse influenza A viruses**

Suxiang Tong1, Xueyong Zhu5, Yan Li1, Mang Shi8, Jing Zhang1, Melissa Bourgeois2, Hua Yang2, Xianfeng Chen1, Sergio Recuenco3, Jorge Gomez10, Li-Mei Chen2, Ying Tao1, Cyrille Dreyfus5, Wenli Yu5, Ryan McBride6, Paul J. Carney2, Amy T. Gilbert3, Jessie Chang2, Zhu Guo2, Charles T. Davis2, James C. Paulson5,6, James Stevens2, Charles E. Rupprecht3,4, Edward C. Holmes8,9, Ian A. Wilson5,7, Ruben O. Donis2

1Division of Viral Diseases,2Influenza Division, 3Division of High Consequence Pathogens and Pathology, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA.

4Global Alliance for Rabies Control.

5Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037, USA.

6Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA 92037, USA.

7Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, CA 92037, USA.

8Sydney Emerging Infections and Biosecurity Institute, School of Biological Sciences and Sydney Medical School, The University of Sydney, Sydney, NSW 2006, Australia.

9Fogarty International Center, National Institutes of Health, Bethesda, MD 20892, USA.

10Direccion

General de Epidemiologıa, Ministerio de Salud - MINSA, Lima, Peru.

Aquatic birds harbor diverse influenza A viruses and are a major viral reservoir in nature. Recent discovery of a subtype H17N10 influenza viruses in Central American fruit bats suggests that other New World species may carry divergent influenza viruses. Using consensus degenerate RT-PCR, we identified a novel influenza A virus designated as H18N11 in a flat-faced fruit bat (Artibeus planirostris) from Peru. Serologic studies with the recombinant H18 protein indicated that several Peruvian bat species were infected by this virus. Phylogenetic analyses demonstrate that New World bats maintain as much influenza virus genetic diversity as all other mammalian and avian species combined, indicative of an ancient host-virus association. Structural and functional analyses of the hemagglutinin and neuraminidase indicate that sialic acid is not a ligand for virus attachment nor a substrate for release, suggesting a novel mechanism of influenza A virus attachment and activation of membrane fusion for entry into host cells. Taken together, these findings indicate that bats constitute a new, likely ancient, and potentially important reservoir for a diverse pool of influenza viruses.

324 **Sever fever with thrombocytopenia syndrome in Japan**

Masayuki Shimojima1, Toru Takahashi2, Shuetsu Fukushi1, Hideki Tani1, Tomoki Yoshikawa1, Tetsuya Mizutani3, Shigeru Morikawa4, Masayuki Saijo1, Ken Maeda5
1Department of Virology I, National Institute of Infectious Diseases, Tokyo, Japan;, 2Department of Hematology, Yamaguchi Grand Medical Center, Yamaguchi, Japan; 3Research and Education Center for Prevention of Global Infectious Diseases of Animals, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Tokyo, Japan; 4Department of Veterinary Science, National Institute of Infectious Diseases, Tokyo, Japan; 5Laboratory of Veterinary Microbiology, Joint-Faculty of Veterinary Medicine, Yamaguchi University, Yamaguchi, Japan

Sever fever with thrombocytopenia syndrome (SFTS) is characterized by sudden onset of fever, leukopenia, thrombocytopenia, and gastrointestinal tract symptoms and approximately 12% of patients die from disseminated intravascular coagulation and/or multiple organ failures. Agent of the disease is a novel bunyavirus SFTS virus, and is transmitted by bite of a possible vector tick, Haemaphysalis longicornis, and through direct contact with virus-containing patient body fluids, or through unknown routes. SFTS case reports have been limited in China, and more than two thousand cases were reported in 2011 and 2012. In late 2012, a woman living in Yamaguchi prefecture in Japan showed symptoms reminiscent of those of SFTS and died 6 days after the onset of symptoms. Virus was isolated from her acute serum in Vero cells and a next generation-sequencing identified it as SFTS virus. SFTS viral genome and proteins were detected in the patient's serum. Based on the first demonstration of SFTS in Japan, a retrospective study started. Until March of 2013, totally 8 patients were diagnosed as having SFTS and the most early case was in 2005. Phylogenetic analysis of virus sequences revealed that Japanese isolates form an independent branch distinct from Chinese isolates, indicating that SFTS has been present not only in China but also in Japan.

325 **Recent Emergence and Spread of a Phylogenetic Lineage of Rabies Virus in Nepal**

Ganesh R. Pant1, Rachel Lavenir2, Frank Y. K. Wong3, Andrea Certoma3, Florence Larrous2, Dwij R. Bhatta4, Hervé Bourhy2, Vittoria Stevens3, Laurent Dacheux2
1 Rabies Vaccine Production Laboratory, Tripureshwor, Kathmandu, Nepal.
2 Institut Pasteur, Unit Lyssavirus Dynamics and Host Adaptation, National Reference Centre for Rabies, WHO Collaborating Centre for Reference and Research on Rabies, 25 rue du Docteur Roux, 75015, Paris Cedex 15, France.
3 Australian Animal Health Laboratory, CSIRO Animal Food and Health Sciences, 5 Portarlington Road, Geelong VIC Australia 3220.
4 Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal

Rabies is a zoonotic disease that is endemic in many parts of the developing world, especially in Africa and in Asia. However its epidemiology remains largely unappreciated in much of these regions, such as in Nepal, where limited information is available about the spatiotemporal dynamics of the main etiological agent, the rabies virus (RABV). In this study, we describe for the first time the phylogenetic diversity and evolution of RABV circulating in Nepal, as well as their geographical relationships within the broader region. A total of 24 new isolates obtained from Nepal and collected from 2003 to 2011 were full-length sequenced for both the nucleoprotein and the glycoprotein genes, and analysed using neighbour-joining and maximum-likelihood phylogenetic methods with representative viruses from all over the world, including new related RABV strains from neighbouring or more distant countries (Afghanistan, Greenland, Iran, Russia and USA). Despite Nepal's limited land surface and its particular geographical position within the Indian subcontinent, our study revealed the presence of a surprising wide genetic diversity of RABV, with the co-existence of three different phylogenetic groups: an Indian subcontinent clade and two different Arctic-like sub-clades within the Arctic-related clade. This observation suggests at least two independent episodes of rabies introduction from neighbouring countries. In addition, specific phylogenetic and temporal evolution analysis of viruses within the Arctic-related clade has identified a new recently emerged RABV lineage we named as the Arctic-like 3 (AL-3) sub-clade that is already widely spread in Nepal.

326 **Investigations of Marburgvirus Spillover from Natural Populations of Rousettus Aegyptiacus**

Jonathan S. Towner1, Brian R. Amman1, Serena A. Carroll1, Tara K. Sealy1, Stuart T. Nichol1, Pierre E. Rollin1 and the Filovirus Bat Ecology Team2-9
1Special Pathogens Branch ,2Infectious Disease Pathology Branch ,3Global AIDS Program, 4Biotechnology Core Facility, Centers for Disease Control and Prevention, Atlanta, USA ; 5National Institute of Communicable Diseases, Special Pathogens Unit, Johannesburg, South Africa; 6WHO, Geneva, Switzerland; 7Uganda Virus Research Institute, Entebbe, Republic of Uganda;8Uganda Wildlife Authority, 9Ministry of Health, Kampala, Republic of Uganda

Marburg hemorrhagic fever (MHF) causes high fatality disease in humans. During 2007 and 2008, four separate episodes of MHF occurred at Kitaka mine and Python cave in southwestern Uganda resulting in two deaths. The common link between these outbreaks was exposure to the Egyptian fruit bat (Rousettus aegyptiacus) which has been subsequently shown to be a reservoir host of Marburg virus and Ravn virus (genus Marburgvirus), based upon RT-PCR, virus isolation, and immunohistochemical data. Python Cave resides within the confines of a protected and readily accessible National Park and provided an ideal opportunity to conduct a multi-year ecological investigation of natural marburgvirus circulation within the bat colony. The data from this study show diverse marburgvirus lineages constantly circulating among R. aegyptiacus bats throughout the year. Further, the data show predictable seasonal fluctuations in the percentage of PCR+ bats within specific age cohorts that appear to forecast times of greater public health risk when compared to the dates of previous marburgvirus spillover events. Interestingly, in late 2012, a fifth MHF outbreak occurred in villages not far from Kitaka mine that may have begun during a predicted season of increased risk. Finally, in an effort to better understand the mechanisms of virus replication, transmission and control in a marburgvirus natural reservoir host, a captive breeding colony of R. aegyptiacus bats was established at CDC. Animals from this colony are being used for experimental infection studies under controlled conditions.

327 **Influenza virus reassortment is highly efficient in the absence of segment mismatch**

Nicolle Marshall1,2, Lalita Priyamvada1,2, Ende3,4, John Steel1, Anice Lowen1
1Department of Microbiology and Immunology, Emory University School of Medicine; 2Microbiolo gy and Molecular Genetics Graduate Program, Emory University; 3Emory Vaccine Center at Yerkes National Primate Research Center, Emory University; 4Immunology and Molecular Pathogenesis Graduate Program, Emory University, Atlanta, Georgia, United States of America

Reassortment is fundamental to the evolution of influenza viruses and plays a key role in the generation of novel epidemic and pandemic strains. Previous studies indicate that reassortment is restricted by segment mismatch, arising from functional incompatibilities among the components of two viruses. Additional factors that dictate reassortment efficiency remain poorly characterized. To examine these additional factors, we developed a system for studying reassortment in the absence of segment mismatch. Silent mutations were introduced into A/Panama/2007/99 virus such that high-resolution melt analysis could be used to differentiate all eight segments of the wild-type and the silently mutated variant virus. The use of phenotypically identical parent viruses ensured that all progeny were equally fit, allowing measurement of reassortment without selection bias. Using this system, we found that reassortment occurred efficiently (88.4%) following high multiplicity infection, suggesting that the process is not appreciably limited by intracellular compartmentalization. That co-infection is the major determinant of reassortment efficiency in the absence of segment mismatch was confirmed by an exponential relationship between the frequency of reassortant viruses and that of co-infected cells. The number of reassortant progeny shed from co-infected guinea pigs was likewise dependent on dose: with 103 and 106 PFU inocula, respectively, 30% and 59% of isolates were reassortants. The introduction of a delay between infections allowed definition of time windows during which super-infection led to reassortment in culture and in vivo. Overall, our results indicate that reassortment between two like influenza viruses is efficient but also strongly dependent on dose and timing.

328 **Investigating the Dynamics of Filovirus Evolution in Cell Culture**

Kendra Alfson, Anthony Griffiths
Texas Biomedical Research Institute

Filoviruses are highly lethal RNA viruses that cause hemorrhagic fever with fatality rates of up to 90%. No approved vaccines or therapies exist for filovirus infections and the viruses must be handled in maximum containment. Evidence suggests that fruit bats may be the natural reservoir for filoviruses but they can infect multiple species. Typically, RNA viruses have high spontaneous mutation rates due to error prone RNA-dependent RNA polymerases. A consequence of high spontaneous mutation and replication rates is populations composed of heterogeneous swarms of related variant sequences, referred to as quasispecies. These swarms have important biological consequences as they allow viruses to evolve rapidly in response to selection pressures. Our preliminary data suggested that filoviruses have mutation rates similar to other RNA viruses. However, the dynamics of filovirus evolution are poorly understood and little is known about the quasispecies present in filovirus populations. We have employed deep sequencing to assess the genetic changes associated with filovirus passage in cell culture. These studies were performed using cultured cells derived from different host species and relatively diverse filoviruses. We have observed interesting changes in the consensus sequence and the quasispecies populations. It is likely that the observed genomic changes can be correlated to phenotype and function. Our data suggest that filoviruses exhibit high genome plasticity and are able to rapidly evolve to different environments. This could have major implications for future filovirus research on emergence, virulence, drug-resistance, and vaccine-development.

329 **Evolution of parainfluenza virus 5**

Bert Rima1, Derek Gatherer2, Hanna Norsted3, Andrew Davison2 and Richard Randall3
1 Centre for Infection and Immunity, School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, Belfast BT9 7BL Northern Ireland , 2 MRC - University of Glasgow Centre for Virus Research, Glasgow G11 5JR, 3 School of Biology, University of St Andrews, BMS Building, St Andrews KY16 9ST, UK

The genome sequences of 11 parainfluenza virus 5 (PIV5) isolates were determined by deep sequencing. In a maximum likelihood phylogenetic tree, 4 canine isolates and a porcine isolate grouped into one clade, and a simian isolate and 5 human isolates fell into a second clade, which had the previously sequenced "cryptovirus" as an outlier. No two isolates differed in sequence by more than 3%, and transitions outnumbered transversions by 6:1. Host-specific mutations were identified, but selection pressures determined from pairwise comparisons of dN/dS ratios were weak or absent. No consistent signal for recombination was detected. The most divergent regions are those encoding the C-terminus of the N protein, the M-F intergenic region and the SH gene? Two canine isolates and the porcine isolate had mutations in the SH coding region and did not express SH. Deep sequencing of the quasispecies in multiple passages of one isolate demonstrated that variants appeared or disappeared on passage, and also allowed the identification and quantification of defective interfering RNA molecules. Despite remarkable conservation among PIV5 isolates from different hosts, the ability of PIV5 to mutate was demonstrated from the high levels of reversion of stop codon-introducing mutations in recombinant viruses lacking expression of the cysteine-rich C-terminal part of V. Thus, in common with other members of the subfamily Paramyxovirinae, PIV5 displays remarkable genome conservation despite having a wide host range and being able to mutate when placed under selection pressure. This study manifests the unexplained constraints on PIV5 genome variation, which will be discussed.

330 **Importin-α3 Restricts Influenza Virus Replication in the Mammalian Respiratory Tract**

Swantje Thiele(1), Debby van Riel (2), A.D.M.E. Osterhaus (2), Gülsah Gabriel (1)
(1)Heinrich-Pette-Institute, Leibniz Institute for Experimental Virology, Hamburg, Germany
(2)Department of Virology, Erasmus Medical Center Rotterdam, Netherlands

Components of the nuclear import machinery, the importin-α proteins, play a crucial role in influenza virus host adaptation and pathogenicity. It has been shown that a switch from importin-α3 (inhibitor of viral polymerase activity) to importin-α7 (activator of viral polymerase activity) usage takes place upon avian-mammalian adaptation. To understand the selective pressure which drives the switch from importin-α3 to importin-α7 dependency upon avian-mammalian adaptation, we have determined the mRNA expression levels of individual importin-α isoforms throughout the mammalian lung. Here, we show that importin-α3 is the most abundantly expressed isoform with up to 4-times higher levels than importin-α1, -α4, -α5 or -α7 in the murine and human respiratory tract. Studies in mice revealed that importin-α3 is up-regulated upon infection with avian-like (PB2 701D, NP 319N) and down-regulated with mammalian-like (PB2 701N, NP 319K) influenza viruses. Furthermore, up-regulation of importin-α3 in the mammalian lung correlated with restricted virus growth while down-regulation of importin-α3 correlated with systemic spread and pathogenicity in mice. Thus, selective down-regulation of the inhibitor of viral polymerase activity (importin-α3) and the adaptation to the activator of polymerase activity (importin-α7) is needed to acquire efficient virus replication in the mammalian respiratory tract. In summary, our data suggest that importin-α levels in the mammalian respiratory tract provide a host selective pressure which drives the evolution of influenza viruses during adaptation to the mammalian host.

331 **Evolutionary analysis of Peste-des-petits ruminants virus**

Pow Murali Muniraju1, Muhammad Munir2, Ashley Banyard3, Jingyue Bao4, Aravindh Babu1, Mana Mahapatra1, Geneviève Libeau5 , Carrie Batten1, Satya Parida1*
1. The Pirbright Institute, UK
2.Swedish University of Agricultural Sciences, Uppsala
3. Animal Health and Veterinary Laboratory Agency, UK
4. China Animal Health and Epidemiology Centre, China
5.CIRAD, France

Peste-des-petits ruminants virus (PPRV) (Family Paramyxoviridae, Genus morbillivirus) causes an economically significant disease in sheep and goats. Phylogenetically, isolates of PPRV group into 4 distinct lineages whilst serologically the response to infection/vaccination is monotypic. Currently, the molecular epidemiology of PPRV is based on sequence data derived from short amplicons from the F and N genes. To date, full genome data are available for several different isolates including: one from lineage I; two from lineage II; and five isolates belonging to lineage IV. Recently we have generated full genome sequence data for two more isolates from lineage IV, circulating in North and East Africa, and three isolates from lineage III from infected animals in the Middle East and East Africa. These latter sequences constitute the first lineage III sequences available. From this novel, and pre-existing data we have performed a full BEAST analysis. Phylogenetically, analysis of full genome data corresponded with analyses using partial sequence data and all isolates grouped according to established lineages. We further investigated evolutionary viral substitution rates and predicted the time to most recent common ancestry (TMRCA) using both relaxed and random clock Bayesian phylogenetics. Our findings predict that PPRV has an evolutionary rate of 7.11 to 7.53×10-4 nucleotide substitutions/site/year. For the first time we have shown that lineage III PPRV is the virus from which the other lineages evolved. Using these computational analyses we conclude that the TMRCA for lineage III PPRV is the mid-19th century, decades before its established detection in 1942 in Nigeria.

332 **Viral factors affecting the transmission of H1N1 influenza virus**

Patricia J. Campbell, Shamika Danzy, Anice C. Lowen, and John Steel
Emory University

The 2009 pandemic virus (pH1N1) spread efficiently among humans, with an estimated 60 million cases of infection occurring between April 2009 and April 2010 in the US. The virus emerged from the swine influenza reservoir, following reassortment of strains from two distinct lineages (N. American TRIG lineage, which donated 6 gene segments to the pandemic strain, and Eurasian "avian-like" swine lineage, which donated the M and NA segments). Interestingly, viruses derived from either lineage are poorly transmissible between humans, raising the question of which viral factors rendered the pH1N1 strain so highly transmissible. To address this question we generated a panel of recombinant viruses in which M, NA, or M and NA segments from pH1N1 or avian-like Eurasian swine strains were introduced into the laboratory-adapted strain A/PR/8/34 (PR8), and tested their fitness in vitro and in vivo. Viruses possessing the NL602 M segment had higher peak growth than those possessing the Eurasian lineage M, in vivo (guinea pig). Furthermore, the virus possessing the NL602 M segment readily transmitted between guinea pigs by contact, while the Eurasian M segment -possessing virus was characterized by slower and less efficient transmission. Analysis of the viruses further indicated that both morphology and neuraminidase activity were altered upon substitution of the M segment. Understanding the role of the viral factors contributing to influenza virus transmission in human hosts will increase our basic understanding of influenza virus biology, and be of value in the identification of influenza strains with pandemic potential.

333 **Genomic analysis of filoviruses associated with 4 hemorrhagic fever outbreaks in Uganda and the Democratic Republic of Congo in**

Cesar Albariño, Trevor Shoemaker, Marina Khristova, Stephen Balinandi, Alex Tumusiime, Shelley Campbell, Deborah Cannon, Aridth Gibbons, Eric Bergeron, Brian Bird, Kimberly Dodd, Christina Spiropoulou, Bobbie Erickson, Lisa Guerrero, Barbara Knust, Stuart Nichol, Pierre Rollin, Ute Ströher
Centers for Disease Control and Prevention, Atlanta, USA

In 2012, an unprecedented number of 4 distinct, partially overlapping filovirus-associated hemorrhagic fever outbreaks were detected in eastern Africa. Analysis of virus complete genome sequences confirmed the reemergence of Sudan virus and Marburg virus in Uganda, and the first emergence of Bundibugyo virus in the Democratic Republic of Congo.

334

Intrapatient Variation of the Respiratory Syncytial Virus Attachment Protein Gene in patients through time

Mariana Viegas12, Ana Julia Velez Rueda13, Alicia S. Mistchenko13
1. Virology Laboratory, Dr. Ricardo Gutiérrez Children's Hospital, Buenos Aires, Argentina.
2. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ciudad Autónoma de Buenos Aires, Argentina
3. Comisión de Investigaciones Científicas (CIC), Provincia de Buenos Aires, Argentina

The human respiratory syncytial virus (HRSV) is the leading cause of acute lower respiratory infections (LRI) in children under five years worldwide and reinfections occur throughout lifetime. The most diverse antigenic and genetic differences between strains are located in the attachment G protein. We analyzed the genetic variation and evolutionary dynamics of the G gene ectodomain in nasopharyngeal aspirates taken from hospitalized patients with LRI during 2011-2012 outbreaks. Three of them suffered prolonged LRI or reinfections and presented two samples which were separated by at most 135 days and were analyzed at intrapatient variation level. Calculation of mean genetic distances (MGD) and Bayesian phylogenetic analyses were performed to analyze the viral population dynamics within samples and to determine relationships between local and global HRSV strains. Patient 1 presented a strong immunesuppression, and as consequence viral variants within the first and the second sample (50 days apart) clustered together with their direct sequences and with each other in a closely related genetic clade, with a maximum MGD of 0.6% (SE 0.1%), thus suggesting a prolonged shedding. Patient 2 harbored in his first sample only viral variants associated with the HRSV strain with a 72-nt duplication that emerged in 2011, but in his second sample (50 days apart) there were at least four types of viral variants associated with the first sample and with local circulating strains and with a maximum MGD of 6.4% (SE0.6%), suggesting a reinfection and a prolonged shedding. And patient 3 suffered his first LRI in the 2011-outbreak and the second in the 2012-outbreak, and the viral variants found between them were completely different, thus suggesting a reinfection. Important conclusions about how the virus encounters and surmounts different immune environments are derived from this study, showing that these patients, in different natural conditions of infection, might act as genetic reservoirs that could provide in turn molecular plasticity to HRSV.

335

Mapping the steps required for the adaptation of animal morbilliviruses to human Cells

Martin Ludlow, W. Paul Duprex
Department of Microbiology, Boston University School of Medicine, Boston, MA 02118 USA

Fundamental studies which dissect the molecular mechanisms governing zoonotic transmission of viruses are vital given the continual bombardment of the human population by animal pathogens. Morbilliviruses provide a safe model system to study cross-species infections as although they are highly infectious respiratory pathogens, they are similar enough for the measles virus (MV) vaccine to provide cross-protective immunity against animal morbilliviruses such as canine distemper virus (CDV). With the public health community currently considering global eradication of measles, it prudent to assume that this may result in significant drops in measles vaccine uptake resulting in a possible risk of zoonotic morbillivirus infections. We developed an *in vitro* model system to study CDV adaptation to human (h) CD150, the canine (c) version of which serves as the primary *in vivo* cellular receptor. A fluorescent recombinant (r) wild-type strain of CDV (rCDVR252EGFP) was used to monitor infection in human B-cells (B LCL) cultures for >150 days. A number of unique mutations were detected in the CD150 binding region of the hemagglutinin (H) glycoprotein with differences observed in the constellation of mutations acquired by hCD150-adapted viruses in six independent replicates and the ability of the resulting virus to use cCD150. These experiments show that morbilliviruses can follow multiple routes to adapt to hCD150 usage and more broadly that gain of function can concomitantly lead to a modulation in the ability of the virus to use its original cellular receptor. This has important implications for our understanding of cross-species viral infections.

336 **Characterization of Influenza A(H3N2)v at County and State Agricultural Fairs in the U.S.**

Rebecca Garten1, Amanda Balish1, Mary Lea Killian2, Sabrina Swenson2, Andrew Bowman3, Charles (Todd) Davis1, William Davis1, Bo Shu1, LaShondra Berman1, John Barnes1, Stephen Lindstrom1, Julie Villanueva1, Alexander Klimov1

1 Centers for Disease Control and Prevention, NCIRD, Influenza Division, Atlanta, Georgia

2 National Veterinary Services Laboratories, United States Department of Agriculture, Ames, Iowa

3 The Ohio State University, Department of Veterinary Preventive Medicine, Columbus, Ohio, USA.

The animal-human interface plays a pivotal role in influenza virus genome reassortment and host-switching of viruses with pandemic potential. Infection in humans with swine-origin viruses (termed variant viruses) has been increasingly detected over the last decade. Between July and September 2012, 308 confirmed human cases of influenza A(H3N2)v virus, possessing the M gene from (H1N1) pdm09, were detected in the United States. The vast majority of cases reported direct contact with pigs exhibited at county or state agricultural fairs. Only five cases had no known exposure to pigs, indicating limited human-to-human transmission. In this study, full/partial genomes of H3N2v viruses collected from humans at four state fairs and 35 county fairs were compared to swine viruses collected from the same fairs or through the USDA National Influenza A Virus in Swine Surveillance Program. Genetic analysis revealed nearly identical sequences of viruses sampled from humans and pigs from the same fair and/or state. Additionally, all but three human viruses had hemagglutinin (HA) genes that fell into a single genetic clade, while the HA genes of viruses from pigs were distributed throughout several H3 clades. Among the H3N2v viruses analyzed, the neuraminidase gene showed the most genetic diversity due to multiple introductions of N2 into the North American swine population. Hemagglutination inhibition assays using post-infection ferret antisera found all viruses to be antigenically similar to pre-pandemic vaccine candidate A/Minnesota/11/2010. This study highlights the necessity for surveillance and collaboration between animal and human health agencies to better understand influenza transmission between swine and humans.

337 **Detection of antibodies to pH1N1 Influenza virus in marine mammals from California in 2009-2012**

Ignacio Mena1, Walter M. Boyce2, Pamela K. Yochem3, Frances M. D. Gulland4, Noelia Moreno1, Daniel Perez5, Ana S. Gonzalez-Reiche5, Brent S. Stewart3, Adolfo García-Sastre1
1.Icahn School of Medicine at Mount Sinai, New York, USA. 2.University of California, Davis, USA. 3.Hubbs-SeaWorld Research Institute, San Diego, California, USA. 4.The Marine Mammal Center, Sausalito, California, USA. 5.University of Maryland, USA.

Marine mammals are in contact with important reservoirs of influenza virus, including aquatic birds and humans. After its emergence in humans in 2009, pandemic H1N1 (pH1N1) influenza virus was isolated from 2 Northern elephant seals (NES, *Mirounga angustirostris*) on the coast of central California in the spring of 2010 (Goldstein et al. 2013, PLOS One, in press). To better understand the extent of pH1N1 infection we have used haemagglutination inhibition assay to analyze serum samples from the three most common marine mammals in California: NES (222 samples), California sea lions (CSL, *Zalophus californianus,* 183 samples) and harbor seals (HS, *Phoca vitulina*, 140 samples). Samples from 2009 through 2012 were obtained from free-ranging individuals as well as stranded animals hospitalized at The Marine Mammal Center (Sausalito, CA). Our results show that pH1N1 spread very rapidly in NES populations, with seroprevalence rising from nearly 0% in the spring of 2010 to almost 50% in 2011. Pups from seropositive mothers had pH1N1 specific antibodies, with titers often higher than those of their mothers, but a few 16 month old juveniles sampled in 2012 were seronegative, suggesting that the epizootic had a limited duration and the virus might not be circulating in NES anymore. Contrary to NES, CSL and HS had low prevalence (less than 10%) of pH1N1 specific antibodies. This could be explained by differences in susceptibility to influenza infection, by specific adaptive mutations acquired in the NES virus strains and/or by more efficient intra-species transmission related to animal behavior.

338 **RNA polymerase II-dependent single infectious RABV cDNAs.**

Alexander Ghanem, Karl-Klaus Conzelmann
Genecenter & Max von Pettenkofer-Institute, LMU Munich

The classic rabies virus (RABV) reverse genetics system requires intracellular co-expression of a set of three viral "helper" proteins (N, P, and L) and of correctly (end-) processed T7 RNA polymerase-derived viral RNAs. In order to evaluate the possibility of genetically encoded RABVs, in a first step single infectious RABV cDNAs were generated by the incorporation of picornaviral IRES elements upstream of the N-, P-, and L-ORF of the full-length cDNA. This allowed the direct translation of these "helper"-proteins from the T7 RNA-transcribed antigenome-like RNA and to rescue RABV from a single plasmid. In order to allow T7 RNA polymerase-independent rescue, the suitability of polymerase II promoters was evaluated. Initial approaches, however, failed, due to poor helper protein expression. The correct processing of the 5'- and 3'- termini, while being crucial in regard to rescue efficiency, was observed to interfere with RNA polymerase II-dependent protein expression. This problem was solved by the application of less efficient ribozymatic processing of viral RNA termini on one hand and less attenuated IRES-comprising RABV variants on the other hand. The fast and efficient RNA polymerase II-dependent rescue of RABV from single plasmids may open the way to the development of animal models in which RABV is genetically encoded and an infectious cycle is induced in specific cell types. Especially for neurotracing, but also pathogenicity studies, such systems might contribute to a gain of specificity and accessibility in regard to target cells. They could benefit from the virtual infinite number of genetic mouse models available.

339 **Molecular epidemiology of Crimean Congo Hemorrhagic Fever in Iran**

Sadegh Chinikar,Nariman Shah-Hosseini,Sahar Khakifirouz,Tahmineh Jalali,Bahareh Moghassemi,Tahereh Mohammadi,Mahboubeh Rafigh,Ghazaleh Dadashizadeh *Arboviruses and Viral Haemorrhagic Fevers Laboratory (National Ref. Lab), Department of Virology, Pasteur Institute of Iran*

Background: Crimean Congo Haemorrhagic Fever (CCHF) caused by CCHF virus, belongs to Nairovirus genus and Bunyaviridae family. The route of transmission is through the bite of infected ticks, handling of infected blood or organs of livestock and nosocomially. The virus has a negative and three segments (S, M and L) RNA genome. The initial phylogeny study on CCHFV in Iran showed that isolated Iranian strain (ArTeh193-3) was similar to Senegalese strain. Methods: The viral RNA was extracted from human sera (2004 and 2012) and ticks in (2008). The virus genome was examined by RT-PCR. PCR products were sequenced and sequences were analyzed by Mega software.
Results: Our earlier phylogenetic analysis of partial S-segment nucleotide sequences in Iran, in 2004, illustrated that the CCHFV isolates were clustered with strains from Pakistan. These data also demonstrated that the Iranian examined isolates and the previously published CCHFV strain ArTeh193-3 clustered into different genetic groups, indicating that at least two genetic lineages of CCHFV could be co-circulating in Iran. Further investigations on ticks in Isfahan province in Central Iran, in 2008, revealed that the CCHFV genome was detected in 9% of ticks resident in livestock. Phylogenetic analysis demonstrated that a variant isolate was clustered with the Iraq strain. A recent phylogenetic study on the CCHFV genome sequence from the North of Iran in 2012 showed 50% similarity between isolated strain and Russian strains. Conclusion: In our previous studies, CCHF genome S-segment sequences were genetically characterized and results illustrated a close relation with Matin strain (Pakistani Strain). Whereas, our study in 2008 reveals the existence of another strain of CCHF in Iran (Iraqi strain). To date, according to all previous phylogenetic studies, it can be claimed that Senegalese, Pakistani, Iraqi and Russian strains of CCHFV are known to be circulating in Iran.

340 **A formula on the number of amino acid substitutions on the hemagglutinin molecules of H3N2 seasonal influenza viruses**

Kimihito Ito, Manabu Igarashi, Ayato Takada
Hokkaido University Research Center for Zoonosis Control

Human influenza A viruses undergo antigenic changes with gradual accumulation of amino acid substitutions on the hemagglutinin (HA) molecule. A strong antigenic mismatch between vaccine and epidemic strains often requires the replacement of influenza vaccines worldwide. To establish a practical model enabling us to predict the future direction of the influenza virus evolution, relative distances of amino acid sequences among past epidemic strains were analyzed by multidimensional scaling (MDS). We found that human influenza viruses have evolved along a gnarled evolutionary pathway with an approximately constant curvature in the MDS-constructed 3D space. The gnarled pathway indicated that evolution on the trunk favored multiple substitutions at the same amino acid positions on HA. The constant curvature was reasonably explained by assuming that the rate of amino acid substitutions varied from one position to another according to a gamma distribution. Furthermore, we utilized the estimated parameters of the gamma distribution to predict the amino acid substitutions on HA in subsequent years. Retrospective prediction tests showed that about 70% of actual amino acid substitutions were correctly predicted. Although it remains unsolved how to predict the exact timing of antigenic changes, the present results suggest that our model may have the potential to recognize emerging epidemic strains.

341 **Status of immunity against Seoul virus among wild rats (Rattus norvegicus): implications for persistence of Seoul virus**

Kenta Shimizu1, Shumpei P. Yasuda1, Kumiko Yoshimatsu1, Takaaki Koma1, Nguyen Thuy Hoa2, Le Thi Quynh Mai2, Futoshi Hasebe3, Tetsu Yamashiro3, Jiro Arikawa1
1Department of Microbiology, Graduate School of Medicine, Hokkaido University, Sapporo, Japan
2National Institute of Hygiene and Epidemiology, Hanoi, Vietnam
3Nagasaki University, Nagasaki, Japan

Seoul virus (SEOV), one of the serotype of the genus Hantavirus, family Bunyaviridae, is a causative agent of hemorrhagic fever with renal syndrome. SEOV has been maintained within a population of wild rats (*Rattus norvegicus*). However, the mechanism of persistence of SEOV in nature remains unclear. In this study, to examine the relationship between immune response and persistence of SEOV in wild rats, status of host immunity and viral load were investigated. A total of 200 rats were captured in Hanoi and Haiphong, Vietnam in 2009, 2011 and 2012. 44 out of 200 (22.0%) rats were found to be SEOV IgG antibody (Ab) positive, and 37 (84.1%) of which possessed virus genome in lung, suggesting that SEOV had not been cleared efficiently after infection. 26 (59.1%) of IgG Ab positive rats possessed IgM Ab, which was unusually high proportion. In female, negative relationship between IgG avidity and the amount of virus genome in lung was observed. In contrast, there was no relationship between them in male. SEOV specific CTL activity was not detected in SEOV positive rats. These results suggest that impairment of humoral and cell-mediated immunities against SEOV drive persistent infection in natural host.

342 **Characterization of the evolutionary relationships among African orthobunyaviruses**

Allison Groseth, Carla Weisend, Eric Dahlstrom, Sarah L. Anzick, Stephen F. Porcella, Hideki Ebihara *1 Laboratory of Virology, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 903 South 4th Street, Hamilton, MT 59840, USA 2 RML Genomics Unit, Research Technology Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 903 South 4th Street, Hamilton, MT 59840, USA*

Bunyaviruses represent one of the most frequent, but underappreciated, causes of arthropod-borne disease in Africa where they cause febrile illness and in some cases also meningitis/encephalitis or even hemorrhagic complications. However, from a genetic standpoint the family has been only poorly characterized with most virus identification still based on serological testing combined with limited sequencing data, which almost exclusively focuses on the S-segment. As a result the exact identity of many bunyaviruses remains unclear. In order to improve our understanding of the evolutionary relationships between bunyaviruses causing human disease, we have undertaken the complete genome sequencing of various human pathogenic orthobunyaviruses and their close relatives, focusing on African members of the Bunyamwera, Nyando and Bwamba serogroups. These data represent a wealth of molecular information for a variety of viruses for which complete genome sequencing data was previously unavailable, and analysis of this data has identified several viruses whose taxonomic classification needs to be revisited. This includes the identification of viruses that appear to be previously unrecognized reassortants, as well as viruses that have been assigned to a distinct species but would be more appropriately classified as strains of a single species, and viruses that are currently regarded as a single species but for which genetic divergence would support classification as distinct species. These data not only significantly increase the genetic resources available for research pursuits, but will increase our ability to conduct effective surveillance for existing viruses, and to identify and predict the impact of the emergence of novel bunyaviruses.

344 **A simple method for the detection of tick-borne phleboviruses by one-step RT-PCR - a perfect tool for virus hunting**

Keita Matsuno
Molecular Virology and Host-Pathogen Interaction unit, Laboratory of Virology, Division of Intramural Research, Rocky Mountain Laboratories, NIAID, NIH

The public health importance of tick-borne phleboviruses (TBPVs) is increasing, in particular, because two novel TBPVs that are pathogenic for humans have recently emerged: Severe Fever with Thrombocytopenia Syndrome virus (SFTSV) in China and Japan and Heartland virus (HRTV) in the US. These TBPVs comprise a related group within the genus Phlebovirus along with Bhanja viruses (BHAVs), another group of TBPVs pathogenic to humans, and Uukuniemi viruses (UUKVs). Due to strong antigenic and genetic diversity, surveillance and diagnosis of TBPVs has been difficult. We have established a simple and fast detection method for multiple TBPVs using one-step RT-PCR with a single pair of degenerate primers based on conserved regions of the virus genome. Our RT-PCR could amplify multiple TBPVs, including SFTSV, HRTV, BHAVs, and UUKVs. The system could also amplify Lone Star virus and Kisamayo virus and found that they are distantly related to the BHAVs. Also we revealed that two uncharacterized bunyaviruses, Silverwater virus and Lanjan virus compose a novel species distantly related to the UUKV group. Furthermore, we found a completely novel species of TBPV in ticks collected in Mali, tentatively designated Kati virus. In conclusion, our simple RT-PCR system is of sufficient sensitivity and broad-reactivity to detect a wide range of TBPVs, including heretofore undiscovered viruses divergent from known TBPVs. This assay system can help field studies in detection of a wide variety of known and novel TBPVs, and can also be applied to the identification of uncharacterized febrile illnesses associated with tick bites.

345 **Identification of a single point mutation in the 220 loop of equine influenza virus H3 that may play a role in adaptation to dogs**

Adam Rash (1), Alana Woodward (1), Liz Medcalf (1), Emily Holliday (2), Laura Peachey (2), Greg Dowd (2), Janet Daly (3), John McCauley (4), Debra Elton (1). (1) *Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk, CB8 7UU, UK; (2) Royal Veterinary College, North Mymms, Hatfield, AL9 7TA, UK; (3) School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, LE12 5RD, UK; (4) National Institute for Medical Research, Mill Hill, London, NW7 1AA,UK;*

2-3 linkages 2-6 linkages. Equine influenza viruses (EIV) bind receptors with 2-3 or Influenza virusesfrom both horses (H3N8) and birds (H3N2) have crossed the species barrier to infect dogs. One barrier to such transmission events is poor binding to host cell receptors. The viral haemagglutinin (HA) is responsible for binding to specific sialic acid residues on cell surfaces and these vary between animal species. Influenza viruses from different hosts therefore have different receptor preferences, such as specificity for and terminal Neu5Gc rather than Neu5Ac. The receptor preferences of canine influenza viruses (CIV) have yet to be resolved. Specific amino acid changes within the 220 loop of HA alter receptor binding specificity and/or affinity. We compared H3 viruses from birds, horses and dogs and found that CIV of H3N8 and H3N2 subtypes have a specific change within this region. A clinical isolate of equine influenza, which grew better on canine cells than in eggs, identified a different substitution at the same location. Passage of EIV on canine tracheal explants also resulted in this change. Viruses containing the 220 loop mutations were rescued by reverse genetics and their growth characteristics compared with wild type EIV in canine cells and tracheal explants cultures. A mutant with a canine-like 220 loop mutation caused more extensive cell lysis in MDCK cells than the wild-type equine virus and replicated more efficiently in canine tracheal explant cultures. A single mutation in the 220 loop of H3 viruses may therefore be important for adaptation to canine cells.

Abdelwhab, El-Sayed 31, 157
Abdullah 29, 158
Abe, Masako 21, 158
Abreu, Pablo 195
A.C.Shurtleff 94
Acuña, Rodrigo 52
Ader-Ebert, Nadine 53, 60
Adomeh, Donatus I. 33, 169
Aguilar, Hector C. 45
Ahlm, Clas 160
Akeda, Yukihiro 172
Akhuemokhan, Odigie C. 169
Akpede, George O. 169
Albariño, César G. 149,156,216
Albertini, Aurélie A. 47
Albrecht, Randy A. 164, 201
Aldous, Elizabeth 155,161
Alexey, Prilipov 88
Alfonso, R. 104
Alfson, Kendra 214
Alikah, Sylvester O. 169
Aliyari, Roghiyh 136
Allart, Sophie 59
Allen, Adria 45,70,79
Alonso, Jesus 76
Alves, Lisa 60
Alymova, Irina 151
Amada, Takako 167
Amarasinghe, Gaya. K. 110
Amerongen, Geert van 192
Ami, Yasushi 112,209
Amman, Brian R. 213
Ammayappan, Arun 153
ANCHISI, Stéphanie 124,126,141
Andersen, Kristian 169
Anderson, Danielle E. 161
Andrejeva, Jelena 137
Anggakusuma 192
Antonella, Tamara 91
Anzick, Sarah L. 221
A.Page 163
Arai, Tetsuro 170
Archambaud, Mehdi 126
Arck, Petra 114
Arikawa, Jiro 105,167,221
Arranz, Rocío 82
Asenjo, Ana 86
Asensio, Víctor 144
Asogun, Danny A. 169
Atkins, Alex 55
Audsley, Michelle 123
Ayllon, Juan 100,113,179
Babiuk, Shawn 190
Babu, Aravindh 215
Badham, Matthew 55
Baechler, Elodie 187
Baets, Sarah De 203
Bailey, Dalan 51
Baize, Sylvain 137
Bajorek, Monika 54
Baker, Steven F. 201
Bakkouri, Karim El 203
Bales, Jacquelyn M. 153
Balinandi, Stephen 216
Balish, Amanda 194, 218
Balkema-Buschmann, Anne 106
Ballif, Bryan A. 96
Balzarini, J. 191
Bamford, Connor G. G. 62
Bampi, Carole 49
Banadyga, Logan 127
Bance, Nicole 182
Banerjee, Amiya K. 69
Banyard, Ashley 200,205,215
Bao, Jingyue 215
Baquero, Eduard 47
Barclay, Wendy S. 74,138,206
Bardin, Philip G. 55
Barnes, John 218
Baron, Jana 185
Baron, Michael D 98,121,143,185
Barrett, Susan 182
Barry, Gerald 98
Basler, Christopher F. 63,75,110
Batten, Carrie 205,215
Baule, A. 94
Baumgärtner, Wolfgang 98
Bavari, Sina 94,103
Blattmann, B 194
Beaty, Shannon M 70,79,208
Becher, Anne 156
Becker, Stephan 49,66,75,77,121
Becker-Ziaja, Beate 169
Bederka, Lydia 95
Bedwell, Gregory J. 87
Behrendt, Ilona 73
Belicha-Villanueva, Alan 125,164,205
Bengoechea, José A. 144
Bente, Dennis A. 149
Bergeron, Eric 149,216
Berg, Thierry van den 186
Berman, LaShondra 218
Bernardo, Beatriz Sanz 143
Bethel, Laura 153,157
Bétourné, Alexandre 111,146
Beusekom, Ellen van 97
Beyleveld, Grant 179
Bhatta, Dwij R. 212
Bhella, David 50
Bialas, Kristy 53

authors

Bianchi, Matteo 135,194
Biedenkopf, Nadine 66
Biering, Scott B. 45
Bignon, Christophe 64
Bino, Silvia 131
Birch, Jamie 51
Bird, Brian 156,216
Bisaillon, Martin 161
Bi, Zhenqiang 54
Björkström, Niklas 150
Blackledge, Martin 84
Blakqori, Gjon 81
Blocquel, David 64,93
Blomen, Vincent A. 97
Blondel, D. 80,94,103,133,145
Bloyet, Louis-Marie 122,141
Boergeling, Ivonne 179
Boer, Matthijn de 48
Bogaert, Pieter 175,203
Bogs, Jessica 158
Bokhoven, Hans van 97
Bonhomme, Cyrille 95
Bonnaud, Emilie 146
Bontems, François 89
Bosch, Berend Jan 48
Bose, Sayantan 45
Böttcher-Friebertshäuser, E. 120,180
Botten, Jason 96
Bouloy, Michèle 224
Bourgeois, Melissa 211
Bourhy, H. 78,94,126,145,212
Bousse, Tatiana 195
Bovin, Nicolai V. 86
Bowman, Andrew 218
Boyce, Walter M. 219
Boysone, Jonathan 96
Bradel-Tretheway, Birgit 45
Bradley, Konrad 138
Brady, Clare 148
Brancale, A. 191
Brankin, Brenda 148
Brauburger, Kristina 127
Braun, Monika 150
Breithaupt, Angele 158,176
Bressanelli, Stéphane 147
Briand, Francois-Xavier 72
Brice, A. 94,145
Bridges, Olga 207
Brining, Douglas 199
Broadbent, Lindsay 143
Brock, Linda G 148
Brookes, Sharon M. 167
Broome, R. 176
Brown, Ian H. 155,161,167
Brown, Paul A. 72
Bruce, Emily A. 57,61
Bruchez, Anna 56
Brueggemann, Anne 77
Brummelkamp, Thijn R. 97
Brunel, Joanna 64,84
Bublot, Michel 186
Buchholz, Ursula J 132,148,177
Buchini, Sabrina 182
Buchmeier, Michael 95
Buczkowski, Hubert 205
Bukreyev, Alexander 132
Buonocore, Linda 47
Burri, Dominique J. 108
Busch, Laura 128
Bushmaker, Trenton 165
Bussey, Kendra 53
Bu, Zhigao 197
Byrn, Randy 197
Caballero, Monserrat 157
Çağlayık, Dilek Yağcı 131
Caì, Yíngyún 103
Campbell, Patricia J. 216
Campbell, Shelley 156,216
Camporeale, Gabriela 92
Cannon, Deborah 216
Cano, Olga 177
Cant, Andrew J. 125
Caporale, Marco 98
Cara, A. 188
Carabaña, Juan 157
Carette, Jan E. 97
Carney, Paul J. 211
Caroline, Amy 157
Carrascosa, José L. 82
Carroll, Serena A. 213
Carsillo, Thomas J. 192
Carter, Jade 179
Casas, Inmaculada 164
Casasnovas, José M. 142
Castan, Alexandre 161
Cattaneo, Roberto 46,84,210
Cauldwell, Anna 74,206
Dreyfus, C. 194,211
Celma, María L. 142,157
Cendron, Laura 108
Certoma, Andrea 212
Chai, Qing Q. 160
Chakraborti, Ayan K 117
Chalons, Marie 159
Chanda, Sumit K. 195
Chang, Jessie 211
Charlier, Caroline Marie 59
Chelbi-Alix, Mounira K. 133
Chen, Li-Mei 211
Cheng, Benson Y. H. 82,201
Cheng, Genhong 136
Chen, Gong 179
Cheng, Puran 160
Cheng, X. 176
Chen, Hong-Ming 182
Chen, Hualan 187

chen, Pucheng	187
Chen, Xianfeng	211
Chen, Z.	176
Chen, Zhenhai	71
Chepurnov, Alexander	107,130
Chernyy, Evgeny S.	86
Chin, Daniel J.	179
Chinikar, Sadegh	220
Cifuentes, Nicolás	52
Cillóniz, Cristian	179
Clark, Michael	197
Coleman, John	188
Collins, Peter	128,132,148,177
Coloma, Rocío	82
Como, Najada	131
Compans, Richard W.	127
Conzelmann, Karl Klaus	78,123,126,219
Cooper, Ross	155,161
Corman, Victor Max	109
Cornillez-Ty, Cromwell	96
Cosby, S. Louise	148
Cote, Marceline	56
Cotter, C.	176
Courtillon, Celine	72
Cox, Robert	87
Coyle, Peter V.	106,143
Cruz, Jazmina L.G.	134,205
Cuevas, Maite	164
Cui, Sheng	99
Cunningham, James	96
Cusack, Stephen	81,141
Cusi, Maria Grazia	131,204
Czub, Markus	72
Dabundo, Jeffrey	45
Dacheux, Laurent	212
Dadashizadeh, Ghazaleh	220
Dahlstrom, Eric	221
Dallocchio, Roberto	200
D'Antuono, Alejandra	69
Danzy, Shamika	216
Dave, Keyur	132
Davey, Robert	58,95,107
Davies, Ioana	197
Davis, Charles T.	211
Davison, Andrew	138,214
Davis, William	218
DeBuysscher, Blair L.	178
Deeg, Christoph	104
Deflube, Laure	64
Deivanayagam, Champion	87
Delmas, Bernard	119
Delmas, Olivier	78,126
Delpeut, Sebastien	46
Deng, Guohua	187
Deng, Tao	99
Denis, GERLIER	64,84,122,141
Desfosses, Ambroise	80
Dessì, Alessandro	200
Devaux, Patricia	84
Deventhiran, Jagadeeswaran	151
Dhondt, Kevin	159
Dibben, Oliver	193
Dickinson, Paul	125
Diederich, Sandra	61
Diepen, Angela van	114
Dietzel, Erik	60,72
DiNapoli, Joshua	198
Ding, Shujun	54
Dlugolenski, Dan	189
Do, Danh	96
Dodd, Kimberley A.	139,171,186
DOI, TOMOMITSU	118
Dolnik, Olga	49,77
Domi, Arban	188
Doms, Bob	61
Doms, Robert W.	57
Dong, Changjiang	81
Dong, Haohao	81
D ng, Lián	103
Dongo, Andrew	169
Donis, Ruben O.	211
Dornfeld, Dominik	129
Dosnon, Marion	64,84
Douglas, Isobel	106,143
Drexler, Jan-Felix	56,109
Drosten, Christian	56,109
DuBois, Rebecca M.	207
Duprex, Paul	62,70,79,192,208,217
Durand, Eric	93
Dutch, Rebecca E.	62
Dzieciolowski, Julia	145
Easton, Andrew J	200
Ebihara, Hideki	110,127,221
Edinger, Thomas	119,122
Effantin, Gregory	80
Eggink, Dirk	174
Ehiane, Philomena E.	169
Ehimuan, Jacqueline	169
Ehrhardt, Christina	179
Eisenhauer, Philip	96
Eisfeld, Amie J.	152
Ejima, Miho	183
Elankumaran, Subbiah	70,79,151
Elderfield, Ruth	138
ELEOUET, Jean-Francois	190
Eléouët, Jean-François	85,89
Elliott, Richard M.	81
Elson, Andrew	71
Emerman, Michael	207
Ende,	213
Erickson, Bobbie	186,216
Esperante, Sebastian	83,91
Essaidi-Laziosi, Manel	49,90
Esumi, Mariko	112
Eterradossi, Nicolas	72
KOWALINSKI , Eva	141

Evans, Jennifer S	45,200
Faerman, Carlos	197
Failloux, Anna-Bella	149
Falcon, Ana	164
Falzarano, Darryl	165
Fan, Jun	184
Feagins, Alicia	110
Fearns, Rachel	64,71
Fehling, Sarah Katharina	51,59
Feinman, Leighland J.	124
Feldmann, Friederike	199
Feldmann, Heinz	75,165, 166, 178, 196,199
Feng, Qiulin	197
Fernandez-Muñoz, Rafael	157
Fernandez-Sesma, Ana	125
Ferreira, Helena Lage	186
Ferron, François	93
Fiers, Walter	175,203
Finke, Stefan	102,106,166
Fix, Jenna	89,190
Flamand, Marie	149
Flint, Michael	107
Fodor, E	65,77,109
Folarin, Onikepe	169
Foltzer, Michael	156
Fonseca, Wendy	173
Fontana, J.	188
Fooks, Anthony R	200
Foret, Charlotte	146
Foscaldi, Sabrina	69
Fouchier, Ron A.M.	73,114
Fouquet, Baptiste	103
Frace, Mike	156
Freiberg, Alexander N	70,79,158
Frensing, Timo	73
Frenzke, Marie	210
Frías-Staheli, Natalia	89
Fujikura, Daisuke	113
Fujino, Kan	115,136,140
Fujisaki, Seiichiro	183
Fujiyuki, Tomoko	154
Fukuhara, Hideo	112,134
Fukushi, Shuetsu	116,212
Fukuyama, Satoshi	202
Fuller, Chad	155,161
Furuta, Yousuke	181
Futcher, Bruce	67
Gabbard, Jon D.	189
Gabiane, Gaëlle	85
Gabriel, Gülsah	114,171,215
Gabriel, Martin	169
Gaillard, Vanessa	187
Gallo, Mariana	92
Galloux, Marie	85,89
Galloway, Summer E.	165
Gambaryan, Aleksandra S.	86
Gao, Honglei	197
Garcin, Dominique	108,124,141,187
Garten, Rebecca J.	194,218
Garten, W.	51,59,120,180
Gatherer, Derek	138,214
Gaudin, Yves	47
GAULT, Elyanne	190
Gay, Gonzalo de Prat	83,91,92
Geisbert, Thomas W.	110
Ge, Jinying	197
Genzel, Yvonne	73
Gerber, Doron	54
Gerton, Jordan M.	83
Ghanem, Alexander	78,219
Ghazal, Peter	125
Ghildyal, Reena	54,55
Ghimire, Anil	83
Gibbons, Aridth	216
Gilbert, Amy T.	211
Gilbert, Brian	203
Gillespie, Virginia L.	164
Gire, Stephen	169
Glennon, Nicole B	144
Goffic, Ronan Le	169
Gohrbandt, Sandra	158
Gómez, Beatriz	102
Gomez, Jorge	211
Gómez-Medina, Sergio	134
Gommet, Celine	149
Gonzalez-Dunia, Daniel	59,111,146
Gonzalez-Reiche, Ana S.	219
Goodbourn, Stephen	125,137,138,143
Gordian, Schudt	49
Gorman, Jeffrey	132
Gorseth, Elise	184
Goto, Hideo	140,181
Götz, Veronika	93,129
Graaf, Miranda de	114
Gräber, Marcus	158
Granzow, Harald	176
Green, Amanda	151
Green, Todd J.	87
Griffiths, Anthony	214
Grigorov, Boyan	122
Grimes, Jonathan	126
Groppo, Rachel	198
Groseth, Allison	75,121,221
Gruet, Antoine	64,84
Grund, Christian	168,176
Gubareva, Larisa V.	194
Gudheti, Manasa V.	83
GUERRA, Jessica	108,124,141
Guerrero-Juarez, Christian F.	66
Guerrero, Lisa	216
Guillemot, Laurent	149
Guilligay, Delphine	80
Guionie, Olivier	72
Gulland, Frances M. D.	219
Günthard, Huldrych F.	135

Günther, Stephan 162
Guo, Hailong 201
Guo-Parke, Hong 106
Guo, Zhu 194,211
Gupta, Shawon 150,160
Gutsche, Irina 80
G.Withers, Stephen 182
Haan, Cornelis A. M. de 92,164,174,206
Habchi, Johnny 64,93
Habjan, Matthias 128
Hackett, Brent 62
Hackett, Scott 125
Haijema, Bert Jan 92,174
Hai, Rong 111,163
Hale, Benjamin G 111,113,163
Haller, Otto 129,207
Hamada, Nobuyuki 68
Hambleton, Sophie 125
Hangartner, Lars 135,194
Hani'ah 148
Happi, Christian T. 169
Hara, Koyu 68
Hartgroves, Lorian 138
Hartman, Amy 153,157
Hasebe, Futoshi 221
Hasegawa, Hideki 167
Hass, Meike 169
Hastie, Marcus 132
Hatta, Masato 173
Hatta, Yasuko 181
Hattori, Shosaku 154
Hayashi, Tsuyoshi 130
Headlam, Madeleine 132
Heaney, Liam G. 106,143
Heaton, Michael P. 51
He, Biao 71,189
Heijenoort, Carine Van 89
Heldt, Stefan 73
Helenius, Ari 115
Hengrung, Narin 109
Henning, Ann-Kristin 106
Henry, Aurelie 72
Henry, Stephen M. 86
Herbert, Becky 185
Herfst, Sander 114
Héritier, Julien 187
Herold, Susanne 145
Herrler, Georg 56
Herwig, Astrid 75
He, Wen Q. 160
Hicks, Daniel 167
Hikono, Hirokazu 193
Hillyer, Philippa 148
Himeda, Toshiki 187
Hino, Kimihiro 90
Hiono, Takahiro 162
Hippenstiel, Stefan 156
Hirai, Yuya 140
Hoang, Anh Thu Nguyen 160
Hoa, Nguyen Thuy 221
Hocke, Andreas 156
Hodges, Jeffery 83
Hoecke, Silvie Van den 203
Hoegner, Katrin 145
Hoenen, Thomas 75
Hoffmann, F. Michael 181
Hoffmann, Heinrich 193
Hoffmann, Markus 53
Hokke, Corneel H. 114
Holmes, Edward C. 211
Holzer, Barbara 98
Honda, Ayae 133
Honda, Tomoyuki 115,136,140,170
Hongo, Seiji 87
Höper, Dirk 176
Hopfner, Karl-Peter 123
Horie, Masayuki 120,136
Horimoto, Taisuke 181
Horvat, Branka 122,159,183
Hubel, Philipp 128
Huiskonen, Juha T. 65
Hundt, Jana 150
Hunot, Stéphane 111
Hutchinson, EC 65,207
Ibañez, Lorena I. 175
Ichiko, Yurie 199
Ifeh, Veritas E. 169
Igarashi, Manabu 196,199,220
III, John R. Yates 96
Ilyushina, Natalia A. 86
Imai, Masaki 183
Imamura, Yoshihiro 68
Imomeh, Peter 169
Inoue, Atsushi 195
Irie, Takashi 70,142
Irvine, Richard M. 155,161
Ito, Hiroshi 162
Ito, Kimihito 220
Ito, Morihiro 47
Ito, Naoto 94,170
Ito, Toshihiro 162
Iwatsuki-Horimoto, Kiyoko 172,199,202
Jabado, Omar 63,144
Jackson, Dave 50
Jacobson, S. 170
Jade, LOUBER 141
Jae, Lucas T. 97
Jahrling, Peter B. 103
Jalali, Tahmineh 223
Jamin, Marc 80,84
Janosko, Krisztina 103
Jans, David 54,55,94,123,145
Jaramillo, Carlos M. 156
Jardetzky, Theodore S. 45
Jayakody, Buddhika 132
Jensen, Malene Ringkjøbing 84

Jestin, Véronique	72	Klenk, Hans-Dieter	180
Jesus, Paul De	195	Klenk, H.- D.	120
Jiang, Yongping	195	Klimov, Alexander	195,218
Jia, Xue	99	Klingel, Karin	171
Jin, Cong	54	Klingström, Jonas	150,160
Johnson, Patricia	204	Knepper, Jessica	146,156
Johnson, Scott	189	Knippenberg, Ingeborg C. van	81
Jones, Megan E. B.	171	Knoop, Kristeene	95
Jonsdottir, Ingileif	135	Knust, Barbara	156,196
Jorba, Núria	99	Kobayashi, Hirofumi	199
Jordan, Ingo	73	Kobinger, Gary	72
Jorgensen, Erik M.	83	Kochs, Georg	129, 207
Jorquera, Patricia	185	Koenig, Renate	195
Jr, Euripedes A. Ribeiro	80	Koether, Katharina	179
Juelich, Terry L.	158	Kohara, Michinori	154
Juleff, Nicholas	51	Kohl, Alain	98
Kadoi, Koji	133	Kohler, Ines	135,194
Kai, Chieko	78,90,118,154,170	Kojima, Shohei	140
Kainulainen, Markus	128	Kokuho, Takehiko	191
Kajihara, Masahiro	113,196	Kolesnikova, Larissa	49,60
Kalbfleisch, Ted	51	Kolokoltsov, Andrey A.	95
Kalkeri, Raj	197	Komase, Katsuhiro	209
Kallfass, Carsten	104	Koma, Takaaki	167,221
Kameyama, Ken-ichiro	191	Konishi, Misako 18	
Kanou, Kazuhiko	112	Konstantin, Kochergin-Nikitsky	88
Karger, Axel	106	Korchagina, Elena Y.	86
Kariwa, Hiroaki	105	Kortekaas, Jeroen	48,177
Karlin, David	76	Korukluoglu, Gulay	131
Karron, Ruth A.	177	Kota, Majlinda	131
Kashiwagi, Takahito	68	Koudriakova, Elina	81
Kato, Atsushi	112	Krafft, Amy E.	198
Kato, Masahiko	90	Kranzusch, Philip J.	67
Katsura, Hiroaki	172	Kreher, Felix	149
Katze, Michael G.	152	Krüger, Nadine	56
Kawabata, Ryoko	142	Krumm, Stefanie	91
KAWAGUCHI, Atsushi	100	Krzyzaniak, Magdalena	48
Kawaoka, Yoshihiro	50, 152, 172, 173, 181,196, 199, 202	Kubota, Toru	134
		Kühnel, Denis	168
Keil, Günther	106,176	Kuhn, Jens H.	103
Kerkhoven, Ron. M.	97	Kumar, Naveen	105
Khakifirouz, Sahar	220	Kumar, Sandeep RP	151
Khalifa, Youcef Ben	126	Kume, Shinichiro	133
Khosravi, Mojtaba	60	Kunz, Stefan	108, 192
Khristova, Marina	216	Kuraishi, Takeshi	154
Kida, Hiroshi	113,154	Kurath, Gael	153
Kijas, James	51	Kuroda, Kazumichi	113
Killian, Mary Lea	218	Kuroda, Makoto	134
Killip, Marian	138,144	Kurosaki, Yohei	139
Kim, Jin-Hyo	182	Kuusisto, H.V.	145
King, Benjamin	96	Kwasnitschka, Linda	106
Kipper, Sarit	54	Kwit, Krzysztof	155
Kirchhofer, Axel	123	Kwon, Hyun-jeong	154,170
Kishida, Noriko	183	Lahaye, Xavier	103
Kishko, Michael	198	Lalor, Richard	135
Kiso, Maki	181,199,202	Lambrecht, Bart	175
Kiso, Yuri	228	Lambrecht, Bénédicte	186
Klaus, Joseph P.	96	Lamb, Robert A.	45,58,95
Kleanthous, Harry	198	Lamp, Boris	60

Landeras-Bueno, Sara 99
Landesman, Michael B. 83
Larrous, Florence 78,94,145,212
Lasecka, Lidia 121
Lassoued, Safa 89
Laurel, Ryan 66
Lau, Simone 128
Lavenir, Rachel 212
Lawrence, Philip 109,147
Lebreton, Matthew 208
Ledeboer, Mark 197
Ledergerber, Bruno 135
Lee, Benhur 57,70,79,158,208
Lee, Kyungae 56
Leeman, Joshua 197
Leenhouts, Kees 174
Lee, Soo-mi 56
Lefeber, Dirk J. 97
Lemaitre, Evelyne 72
Lemon, Ken 192
leod, Jesica M. Levingston Mac 89
Lepault, Jean 47
Lester, Talia 57
Leung, Daisy W. 110
Levine, Marnie 195
Leymarie, Olivier 119
Leyrat, Cédric 84
Li1, Yan 211
Liang, Mifang 54
Liang, Yuhong 112
Liang, Yuying 105,159
Liao, Lujian 96
Libeau, Geneviève 85,215
Li, Chuan 54
Li, Dexin 54
Liggins, Richard 182
Li, Jiandong 54
Li, Junping 187
Lin, Chao 197
Lindstrom, Stephen 218
Li, Ping 81
Liu, Guoyu 54
Liu, Jinxiong 187
Liu, Qian 45
Liu, Su-Yang 136
Li, Yanbing 187
Li, Zhou 189
Ljunggren, Hans-Gustaf 150
Llompart, C. 104
Llorca, Oscar 82
Lo, Michael K 144,198
Loney, Colin 50
Longhi, Sonia 64,84,93
Long, Jason 229
Lopez, Mary 188
Lopez, Nora 69
Loughran, Sinead 135,204
Lowen, Anice C. 216
Lozach, Pierre-Yves 52
Luco, Sophie 126
Ludlow, Martin 62,217
Lüdtke, Anja 134,205
Ludwig, Stephan 78,179
Lu, J. 176
Luo, Ming 87
Luongo, Cindy 177
Luthra, Priya 110
Lu, Xiaonan 45
Lyall, JW 65
Ly, Hinh 159
Lyons, Jeremy D. 143
Lyons, Michael 79
Maeda, Ken 212
Maenaka, Katsumi 112,134
Maestre, Ana M. 125
Mahapatra, Mana 215
Mai, Le Thi Quynh 221
Maisner, Andrea 56,60,61,72
Makamure, Muriel 66
Makino, Akiko 115,120,140
Maldonado-Pérez, Kenya M. 102
Malet, Hélène 81
Malik, Harmit S. 207
Malnou, Cécile Elisabeth 59
Manicassamy, Balaji 111
Mani, Nagraj 197
Mänz, Benjamin 73,129
Manzoor, Rashid 113,196
MARC, Daniel 88
Markowska-Daniel, Iwona 155
Maroui, Mohamed Ali 133
Marshall, Nicolle 213
Marsh, Glenn A 147
Martín-Benito, Jaime 82
Martínez-Alonso, Mónica 109
Martínez, Máximo B 173
Martínez-Romero, Carles 164,206
Martínez-Sobrido, Luis 82,201
Martorelli, Barbara 131
Marzi, Andrea 196,199
Masic, Aleksandar 190
Mason, Anne 96
Mateo, Mathieu 46,94,163
Mathieu, Cyrille 159,183
Matsuno, Keita 196,222
Matsuyama, Shutoku 112
Mazur, Steven 103
McAuley, Alexander 149
McBride, Ryan 206,211
McCullers, Jon 151
McElroy, Anita K. 171
McFarlane, Melanie 98
McGovern, Naomi 125
McGuigan, C. 191
McKimm-Breschkin, Jennifer L. 182
McLees, Angela 81

McNeil, Colleen 197
McNulty, Shannon 107
McQuaid, Samantha 135,204
McQueen, Nancy L. 70,79
Meinecke, Peter 97
Melero, José A. 144
Melero, Roberto 82
Mena, Ignacio 164,219
Meneghesso, S. 191
Merkler, Doron 162
Messling, Veronika von 72,161
Mettenleiter, Thomas C. 102,150,158,168
Meyer, Daniela 180
Michelini, Z. 188
Miest, Tanner S. 210
Miller, Milagros 184
Mills, John 55
Mire, Chad 110
Misasi, John 56
Mishin, Vasiliy P. 194
Mistchenko, Alicia S. 217
Mitchell, Hugh 152
Mitchell, Patrick S. 207
Miyakawa, Atsushi 170
Miyake, Yukari 133
Miyamoto, Hiroko 113
Mizutani, Tetsuya 212
Moghassemi, Bahareh 220
Mohamad, Siti M.B. 125
Mohammadi, Tahereh 220
Mohan, Gopi S. 127
Moldt, Manuela 123
Momoh, Mojeed 169
Momose, Fumitaka 52
Moncorge, Olivier 206
Monnet, Yann 111
Montano, Javier A. Benavides 45
Mooney, Alaina J. 189
Moormann, Rob 48,177
Moreno, Noelia 219
Morgan, Neil V. 125
Morikawa, Shigeru 116,209,212
Morikawa, Yuko 52
Morin, Benjamin 230
Morrison, Juliet 125
Morrow, Gavin 188
Moscona, Anne 183,204
Moseley, Gregory 94,123,145
M.Ottmann 94
Motz, Carina 123
M, Steck 194
Mueller, Steffen 67
Mühlberger, Elke 127
Müller, Marcel 56
Müller, Marcel A. 103
Munekata, Keisuike 154
Muniraju, Murali 205,215
Munir, Muhammad 215
Munir, Shirin 128
Munster, Vincent J. 165
Muñoz-Alía, Miguel A. 142,157
Muñoz-Fontela, César 134
Muoebonam, Ekene B. 169
Muraki, Yasushi 87
Muramatsu, Mieko 175
Mutz, Pascal 104
Mynn, June 155,161
Naesens, Lieve 191,200
Nagai, Yasuko 162
Nagai, Yoshiyuki 70
NAGATA, Kyosuke 100
Nagata, Noriyo 167
Nair, Vinod 75,89
Najjar, Farah El 62
Nakae, Susumu 170
Nakagawa, Keisuke 170
Nakamur, Shoko 101,115,120,140
Nakao, Momoko 105
Nakatsu, Yuichiro 134
Nakayama, Eri 196
Nakazono, Yoko 68
Nambu, Aya 170
Nash, Bethany 167
Navaratnarajah, Chanakha 46
Navarro, Maria Luisa 86
Negri, D. 188
Nelson, Emily 127
Nemirov, Kirill 94,163
Neumann, Sebastian 106
Newcomb., Laura L. 66
Neyt, Katrijn 175
Ngonda, Saasa 105
Nguyen, Quy 195
Nichol, Stuart T 117,139,149,156,171,213
Nieto, Amelia 104,164
Niewisk, Stefan 183
Niikura, Masahiro 182
Nikitina, Raisa 107
Nikolai, Kaverin 88
Nishimura, Akiko 139
Nishio, Machiko 47,101,140
Nishio, Sanae 167
Nisole, Sébastien 133
Noda, Takeshi 50,196
Nolden, Tobias 166
Norris, Emma 132
Norsted, Hanna 137,214
Nosaka, Tetsuya 47
Noton, Sarah 64,71
Nouën, Cyril Le 148
Noval, Maria Gabriela 91
Noyce, Ryan 46
Núñez, Alejandro 167
Nürnberger, Cindy 104
Nyanguile, Origène 187

Oakley, Katie 185
Ocádiz-Ruiz, Ramón 173
Odagiri, Takato 183
Odia, Ikponmwonsa 169
Odike, Maxy A. C. 169
Oestereich, Lisa 162
Ogino, Tomoaki 69
Oglesbee, Michael 64
Ohara, Yoshiro 87
Ohkura, Takashi 52
Ohta, Keisuke 101,140
Oishi, Kazunori 172
Okada, Kazuma 170
Okadera, Kota 170
Okamatsu, Masatoshi 162
Okamoto, Isao 70
Okogbenin, Sylvanus A. 169
Okokhere, Peter O. 169
Okuwa, Takako 87
Okuzaki, Daisuke 120
Oladapo, Yemisi T. 169
Olejnik, Judith 127
Olivas, Jacqueline 66
Oliveros, Juan C. 144
Olschlager, Stephan 169
Olson, John 80
Oreshkova, Nadia 177
Orozco, Esther 173
Ortín, Juan 82,99,144,164
Ortiz-Riaño, Emilio 82
Orvell, Claes 204
Osterhaus, A.D.M.E. 215
Osunde, Osagie 169
Otsuki, Noriyuki 134
Otte, Anna 171
Ouldali, Malika 47
Oury, Timothy 157
Ozaki, Hiroichi 162
Ozawa, Makoto 173,181,202
Paessler, Slobodan 168,184
Page, Audrey 163
Pala, Nicolino 200
Palermo, Laura 204
Palese, Peter 174,179,195
Palma, Joel Ramos da 108,192
Palmarini, Massimo 98
Palmer, Samantha G 183
Palomo, Concepción 183
Pant, Ganesh R. 212
Panthier, Jean Jacques 149
Papa, Anna 131
Papageorgiou, Nicolas 93
Parida, Satya 205,215
Park, Arnold 57,70,79,158,222
Parks, Christopher 188
Parrington, Mark 198
Parthoens, Eef 129
Pasquato, Antonella 108,192
Patel, Jenish R. 125
Paterson, Duncan 77
Patterson, Jean L. 76
Patzina, Corinna 207
Paulson, James C. 206,207
Peitsch, Catharina 120,180
Pentecost, Mickey 57
Pérez, Beatriz Escudero 109,147
Pérez-Cidoncha, Maite 99,144
Perez, Daniel 111,163,219
Pérez-Girón, José V. 134
Pernet, Olivier 70,79,158,208
Perrault, Jacques 83
Pessi, Antonello 183
Peters, Noel R. 181
Peterson, Charles C. 66
Petric, Martin 182
Peyrin, Jean-Michel 111
Pfaller, Christian K. 126
Phelan, Eric 169
Piao, Zhenyu 172
Pichlmair, Andreas 128
Pickar, Adrian 71
Piedra, Pedro A. 203
Pilling, Pat 182
Piras, Ilaria M. 98
Pirlot, Jean François 186
Pisanelli, Giuseppe 125
Plant, Helen 184
Plattet, Philippe 53,60
Plemper, Richard 91
Pleschka, Stephan 145
Pohl, Marie-Theres 119,122
Pomorska-Mól, Małgorzata 155
Porcella, Stephen F. 221
Porotto, Matteo 183
Powell, Diana S. 153
Power, Patrick 204
Power, Ultan F. 153
Pozo, Francisco 164
Prescott, Joseph B. 165
Pretel, Esteban 83,92
Prevelige, Peter E. 87
Priyamvada, Lalita 213
Purushotham, Sangeetha 87
Pyo, Hyun-Mi 190
Qi, Xiaole 197
Raaben, Matthijs 97,209
Rabilloud, Jessica 122
Rabin, Ronald L 148
Radoshitzky, Sheli R. 103
Rafigh, Mahboubeh 220
Rajsbaum, Ricardo 125
Ramanan, Parameshwaran 110
Raman, Sathya Narayanan Thulasi 218
RAMEIX-WELTI, Marie-Anne 190
Ramirez, Jose 66
Ramp, Kristina 176

Randall, Richard 125,138,144,214
Ratinier, Maxime 98
Rauw, Fabienne 186
Recuenco, Sergio 211
Recuero-Checa, Maria Angeles 82
Reed, Douglas 157
Reeder, DeeAnn 156
Reed, Mark L. 165
Reguera, Juan 81
Reichl, Udo 73
Reid, St Patrick 163
Reimer, Rudolph 114
REMOT, Aude 190
Rennick, Linda J. 62,79,192,205
Resa-Infante, Patricia 82,114
Resende, Ricardo 182
Reynard, Frédéric 186
Reynard, Stéphanie 137
Riblett, Amber 61
Richardson, Christopher 46
Rich, Kirsty 184
Rieger, Toni 162
Riel, Debby van 215
Riemersma, Moniek 97
RIFFAULT, Sabine 190
Rigter, Alan 92,174
Rijnbrand, Rene 197
Rima, Bertus K. 232
Ringel, Marc 60
R.K.Thimmulappa 94
Röder, Anja 102
Roders, Nathalie 133
Rodríguez, Ariel 164
Rodríguez, Laura 34
Rogolino, Dominga 200
Rollin, Pierre E. 156
Römer-Oberdörfer, Angela 102,168,176
Roose, Kenny 203
Roosmalen, Maarten van 174
Rose, John K. 47
Ross, Craig 138
Rossman, Jeremy 55
Ross, Robin A. 67
Rota, Paul A. 156
Rottier, Peter J.M. 92,174,206
Roux, Laurent 49,78,85,90,126
Rueda, Ana Julia Velez 217
Ruedas, John B. 83
Ruigrok, Rob 80,84
Rupprecht, Charles E. 211
Russell, Charles J. 165, 207
Russier, Marion 137
Russo, Joanne 96
Sabeti, Pardis C. 169
Sadykova, Galina 88
Saelens, Xavier 129,175,203
Saffarian, Saveez 83
Safronetz, David 166
Sagara, Hiroshi 50
Saijo, Masayuki 116,209,212
Saito, Takehiko 130
Sakaguchi, Takemasa 70
Sakai, Kouji 112
Sakarai, Yasuteru 95
Sakoda, Yoshihiro 62
Salvatore, M. 183
Samarasinghe, Amali 151
Sanada, Takahiro 105
Sanchez, Abel 1
Sanchez-Aparicio, Maria T 100,124
Sánchez, Mislay Avila 60
Santiago, César 142
Santiago-Olivares, Carlos 102
Santos-Montanez, Jennifer 45
Sastre, Adolfo García 89,111,124, 125 179,201,205,206 219
Sato, Hiroki 78,90
Sato, Yuko 167
Sauerhering, Lucie 61
Sauter, Martina 171
Savellini, Gianni Gori 131
Sawatsky, Bevan 72
Schepens, Bert 129
Scherer, Alexandra U. 135
Schickli, Jeanne H. 177
Schierhorn, Kristina L. 145
Schmid, Richard J. 153
Schmid, Sonja 125
Schmidt, Kristina Maria 127
Schmolke, Mirco 111
Schneider, Brad 208
Schnettler, Esther 98
Schoehn, Guy 80
Schotsaert, Michael 175,203
Schuhmann, Kerstin Monika 123
Schwemmle, Martin 59,93,129
Scott, Dana 165
Sealy, Tara K. 213
Sechi, Mario 200
Sedeyn, Koen 203
Seehusen, Frauke 98
Seekings, James 155,161
Segura, Jean-Manuel 187
Seidah, Nabil G. 108
Seki, Fumio 134,209
Sekizuka, Tsuyoshi 134
Seregin, Alexey 168,184
Seto, Takahiro 105
Shabman, Reed 63,75
Shah-Hosseini, Nariman 220
Sharma, Nishi R. 105
Sharma, Palka 188
Sharpe, Paul 184
Shaw, Megan L 112,124,144,179, 193,195

Shcherbik, Svetlana 195
Shelton, Holly 138
Shevtsova, Anastasia 49,90
Shields, Michael D. 106,143
Shi, Jianzhong 187
Shi, Mang 211
Shimizu, Kenta 167,221
Shimojima, Masayuki 116,181,212
Shirato, Kazuya 112
Shirvastava-Ranjan, Punya 186
Shi, Xiaohong 81
Shoemaker, Trevor 216
Shtanko, Olena 58,107
Shu, Yaoling 64
Singh, Toshna 132
Sizun, Christina 89
Skiena, Steve 67
Sleeman, Katrina 194
Smet, Anouk 203
Smit, Corneel H. 114
Smith, Darren 198
Smith, Derek J. 114
Smith, Jennifer 158
Soda, Kosuke 162
Sofuku, Kozue 115,140
Soubies, Sébastien 104
Soubieux, Denis 88
SOURIMANT, Julien 190
Spann, Kirsten 132
Sparrer, Konstantin 126
Spel, Lotte 48
Spengler, Jessica R. 149
Spiroloulou, Christina 76
SP.Reid 94
Spronken, Monique I. 114
Staeheli, Peter 104
Stech, Jürgen 150
Stech, Olga 158
Steel, John 213,216
Steglich, Constanze 176
Steinhauer, David 165
Steinmann, Eike 192
Steinmetzer, Torsten 180
Stertz, Silke 119,195
Stevaert, Annelies 200
Stevens, James 194,211
Stevens, Vittoria 212
Stewart, Brent S. 219
Stoltz, Malin 150
Stone, Jacqueline A. 45
Strecker, Thomas 59
Streltsov, Victor A. 182
Ströher, Ute 156,216
Sugai, Akihiro 47,78,90
Sugita, Yukihiko 50
Sugiyama, Makoto 170
Suguitan, AL 176
Sundström, Karin 150,160
Sun, Lina 54
Superti-Furga, Giulio 128
Suzuki, Yasuhiko 199
Suzuki, Yasushi 130, 154
Svensson, Mattias 160
Swart, Rik L. de 192
Swenson, Sabrina 218
Syed, Sabriya 46
Szelechowski, Marion 146
Szemiel, Agnieszka M. 81
Taatjes, Douglas 96
Tachedjian, Mary 123
Tahara, Maino 112,209
Takada, Ayato 113,162,175,196,199,220
Takahashi, Toru 212
Takamatsu, Haru-Hisa 121
Takashima, Ikuo 105
Takashita, Emi 183,202
Takeda, Makoto 134,209
Takeuchi, Kaoru 191
Takimoto, Toru 53,199
Tamietti, Carole 149
Tamot, Ninkka 168
Tamura, Tomokazu 162
Tang, Roderick S. 177
Tang, Xiaolin 83
Tani, Hideki 116,212
Tanikawa, Taichiro 130
Tao, Yizhi Jane 80
Tarbouriech, Nicolas 84
Tarnow, Carolin 180
Tashiro, Masato 183
Taylor, Geraldine 185
Taylor, Travis 127
Team, Filovirus Bat Ecology 213
Teifke, Jens P. 158
Tekes, Gergely 67
Teng, Michael N. 55,143
tenOever, Benjamin R. 125
Terrón, M. Carmen 34
Terrosi, Chiara 131
Thiele, Swantje 215
Thieme, René 114
Thompson, Tarin 153
Thouard, Anne 111,146
Tian, Guobin 187
Tian, Meijie 197
Tiley, LS 65
Tiong-Yip, Choi Lai 184
Tischler, Nicole 52,150
Tomini, Eugena 131
Tomonaga, Keizo 115,120,136,140
Tompkins, S. Mark 189
Tong, Suxiang 211
Topaloglu, Haluk 97
Topham, David J. 201
Torre, Juan C. de la 82,201

Touzelet, Olivier	106	Vreede, FT	65
Towner, Jonathan S.	213	Vries, Erik de	164,206
Tran, Kim	54	Vries, Robert P. de	206
Tremaglio, Chadene	64,71	Vries, Rory D. de	192
Trichel, Anita	157	Wallace, Olivia	188
Tripp, Ralph A.	143	Walls, Dermot	204
Tsai, Alice	197	Walpita, Pramila	189
T., Sánchez-Aparicio María	164	Wang, Bingyin	67
Tscherne, Donna M.	164	Wang, Guojun	187
Tsergouli, Katerina	131	Wang, Jianwei	99
Tsuda, Yoshimi	110,113	Wang, Jingfeng	99
Tsunekuni, Ryota	193	Wang, Lin-Fa	147
Tsurudome, Masato	47,101	Wang, Shiwen	54
Tumusiime, Alex	216	Wang, Xianjun	54
Turrell, Lauren	65,109	Wang, Xiaomei	197
Uchida, Yuko	130,154	Wang, Xijun	197
Uebelhoer, Luke	75	Wang, Yao	57,70,79,136
Uemura, Yusaku	68	Ward, Charles	67
Uno, Yukiko	162	Warlich, Sandra	168
Uraki, Ryuta	199,202	Warren, Caroline	159,161
Urata, Shuzo	58,139	Watanabe, Chiaki	130
Usui, Tatsufumi	162	Watanabe, Hiroshi	68
Uyar, Yavuz	131	Watanabe, Shinji	181
Uyigue, Eghosasere A.	169	Waters, Katrina M.	152
Vakharia, Vikram	80,53	Watts, Andrew G.	182
Valappil, Manoj	125	Webby, Richard J.	207
Valpuesta, José M.	82	Weber, Friedemann	81,128
Vanderlinden, E.	191	Weber, Siegfried	150,158
Varela, Mariana	98	Webster, Robert G.	207
Varich, Natalia	88	Weingartl, Hana	119
Vashisht, Ajay	57	Weinheimer, Viola K.	146,156
Vázquez, Mónica	34	Weisend, Carla	221
Veits, Jutta	150,158,176	Weis, Michael	56,61
Velds, Arno	97	Welsch, Jérémy	122
Verburgh, R. Joyce	192	Wennekes, Tom	182
Verhelst, Judith	129	Wen, Zhiyuan	197
Ver, Lorena	34	Werling, Dirk	121
Verma, Vikram	105	Wessels, Marja W.	97
Versantvoort, Hanneke	174	Wessels, Ute	158
Versteeg, Gijs A.	125	Whelan, Sean P. J.	67
V.E.Volchkov	74	White, Kris M.	195
Vidy, Aurore	103	White, Stephen W.	207
Viegas, Mariana	217	Widjaja, Ivy	92
Vigant, Frederic	70,79	Wilson, Ian A.	211
Vijayakrishnan, Swetha	50	Wilson, Michael R.	62
Villanueva, Julie	194,218	Wiltzer, Linda	94,145
Villanueva, Nieves	85	Wimmer, Eckard	67
Villenave, Rémi	106,143	Winter, Christine C	148,177
Vincent, Joel	75	Wit, Emmie de	165
Vito, Ilaria De	204	Witte, Gregor	123
Vloet, Rianka	177	Wohlschlegel, James	57
V.Lotteau	94	Wolfe, Nathan	208
Vodzak, Megan	156	Wolff, Svenja	121
Vogel, Peter	151	Wolff, Thorsten	145,146,156
Volchkova, Valentina A.	74	Wong, Frank Y. K.	212
Volchkov, Viktor E.	74	Wright, Cassandra	132
Vorac, Jaroslav	74	Wright, Edward	200
Vreede, Frank	109	Wright, Kevin	188

Wright, Peter F. 86
Wu, Yuan Ju 59
Wyrzucki, Arkadiusz 135
Xing, Zheng 235
Xu, Hong 183
Xu, Pei 71
Xu, Q. 176
Yabukarski, Filip 84
Yamaguchi, Ryoji 134,209
Yamaguchi, Tsuyoshi 134
Yamamoto, Naoki 162
Yamaoka, Satoko 94,170
Yamashiro, Tetsu 221
Yamauchi, Yohei 115
Yan, Dan 125,138
Yang, Chinglai 127
Yang, Hua 211
Yasuda, Jiro 139,221
Yasuda, Shumpei P. 221
Yasu, Fumihiko 154
Yasugi, Mayo 120
Ye, Jianqiang 111,163
Yochem, Pamela K. 219
Yoneda, Misako 207
Yoon, Sun-Woo 207
York, Ashley 65
Yoshida, Asuka 70,142
Yoshida, Reiko 113,175,196,199
Yoshii, Kentaro 105
Yoshikawa, Tomoki 116,212
Yoshimatsu, Kumiko 105,167,221
Young, Dan F. 125
Ysenbaert, Tine 175
Yüksel, Selma 192
Yumine, Natsuko 140
Yun, Nadezhda E. 168,184
Yun, Tatyana 158
Yu, Qin 184
Yú, Shuǐqìng 103
Yusuff, Shamila 153
Yu, Wenli 211
Zagordi, Osvaldo 135
Zamarreño, Noelia 82
Zanotti, Giuseppe 108
Zaraket, Hassan 207
Zell, Roland 129
Zengel, James 71
Zeng, Xianying 187
Zhang, Bin 96
Zhang, Jing 211
Zhang, Lihong 158
Zhang, Quanfu 54
Zhāng, Téngfēi 103
Zhang, Xinsheng 188
Zhao, Lili 99
Zhao, Shuangcheng 187
Zheng, Chunlei 96
Zheng, Wenjie 80
Zhou, Changhong 198
Zhou, Hongbo 206
Zhou, Yan 190
Zhou, Zhuo 99
Zhu, Liqian 105
Zhu, Xueyong 211
Ziller, Mario 176
Zimmermann, Petra 129
Zoppi, Florian 192
Zurbriggen, Andreas 53,60

Aguilar,Hector C	Washington State University,haguilar@vetmed.wsu.edu
Albarino,Cesar	Centers for Disease Control and Prevention ,bwu4@cdc.gov
Albarino,Cesar	Centers for Disease Control and Prevention, Atlanta, USA,calbarino@cdc.gov
Alonso,Jesus	Texas Biomedical Research Institute,jalonso@hcc-nd.edu
Alymova,Irina	St. Jude Children's Research Hospital,irina.alymova@stjude.org
ANCHISI,Stéphanie	Département Microbiologie & Médecine Moléculaire - CMU Genève,Stephanie.Anchisi@unige.ch
Anderson,Danielle	Duke-NUS Graduate Medical School,danielle.anderson@duke-nus.edu.sg
Andrejeva,Lena	University of St. Andrews, School of Biology,ja21@st-andrews.ac.uk
Arikawa,Jiro	Hokkaido University Graduate School of Medicine,j_arika@med.hokudai.ac.jp
Audsley,Michelle	Monash University,michelle.audsley@monash.edu
Avila Sánchez,Mislay	Division of Neurological Sciences, Vetsuisse Faculty, University of Bern,mislay.avila@vetsuisse.unibe.ch
Ayllon Barasoain,Juan	Department of Microbiology and Global Health and Emergent Pathogens Institute, Icahn School of Medicine at Mount Sinai,juan.ayllon@mssm.edu
Bailey,Dalan	The Pirbright Institute/ The University of Birmingham,dalan.bailey@gmail.com
BAIZE,Sylvain,	INSTITUT PASTEUR - UBIVE,sylvain.baize@inserm.fr
Bajorek,Monika,	Imperial College London,m.bajorek@imperial.ac.uk
Baker,Steven,	University of Rochester,steven_baker@urmc.rochester.edu
BAMFORD,CONNOR	QUEEN'S UNIVERSITY, BELFAST,cbamford01@qub.ac.uk
Banerjee,Amiya	Professor Emeritus,banerja@ccf.org
Bankamp,Bettina	Centers for Disease Control and Prevention,bbankamp@cdc.gov
Bankwitz,Dorothea	DB,dorothea.bankwitz@twincore.de
Banyard,Ashley	AHVLA,ashley.banyard@ahvla.gsi.gov.uk
Barclay,Wendy	Imperial College London,w.barclay@imperial.ac.uk
Baron,Michael	The Pirbright Institute,michael.baron@pirbright.ac.uk
Barry,Gerald	University of Glasgow,gerald.barry@glasgow.ac.uk
Basler,Christopher	Icahn School of Medicine at Mount Sinai,chris.basler@mssm.edu
Beaty,Shannon	University of California, Los Angeles,smbeaty@ucla.edu
Becker,Stephan	Institut fuer Virologie, Philipps-Universitaet Marburg,becker@staff.uni-marburg.de
Beilstein,Frauke	CNRS LVMS,frauke.beilstein@vms.cnrs-gif.fr
Bergeron,Eric	Centers For Disease Control and Prevention,ebergeron@cdc.gov
Bergeron,Éric	Centers for Disease Control and Prevention,ebergeron@cdc.gov
BIACCHESI,Stéphane	INRA,stephane.biacchesi@jouy.inra.fr
Bianchi,Matteo	University of Zurich,bianchi.matteo@virology.uzh.ch
Biedenkopf,Nadine	Institute of Virology, Philipps-University Marburg,nadine.biedenkopf@staff.uni-marburg.de
Bird ,Brian	Centers for Disease Control and Prevention,che3@cdc.gov
BLOCQUEL,David	AFMB, UMR7257, CNRS and Aix-marseille university,david.blocquel@afmb.univ-mrs.fr
Blondel,Danielle	CNRS,danielle.blondel@vms.cnrs-gif.fr
Bloyet,Louis-Marie	INSERM,louis-marie.bloyet@inserm.fr
Böttcher-Friebertshäuser,Eva	Institute of Virology, Philipps-University Marburg,friebertshaeuser@staff.uni-marburg.de

Bourhy,Hervé	Institut Pasteur,herve.bourhy@pasteur.fr
Bousse,Tatiana	Center for Disease Control and Prevention,tbousse@cdc.gov
Bradley,Konrad	Imperial College London,k.bradley@imperial.ac.uk
BREMONT,Michel	INRA,michel.bremont@jouy.inra.fr
Broadbent,Lindsay	Queens University Belfast,lbroadbent01@qub.ac.uk
Brown,Paul	ANSES ploufragan,paul.brown@anses.fr
Bruce,Emily	University of Pennsylvania,embruce@gmail.com
Brueggemann Anne	Institute of Virology, Philipps University Marburg, Marburg, Germany,anne.brueggemann@staff.uni-marburg.de
BRUNEL,JOANNA	CIRI - INSERM U1111,joanna.brunel@inserm.fr
Brunotte,Linda	University Hospital Freiburg,linda.brunotte@uniklinik-freiburg.de
Buchholz,Ursula	NIAID, NIH,ubuchholz@niaid.nih.gov
Buchmeier,Michael	University of California, Irvine,m.buchmeier@uci.edu
Bucht,Goran	FOI, Swedish Defence Research Agency,goran.bucht@foi.se
Buczkowski,Hubert	AHVLA,hubert.buczkowski@ahvla.gsi.gov.uk
Cattaneo,Roberto	Mayo Clinic,higgins.toni@mayo.edu
CHARLIER,Caroline	INSERM U1043, Centre de Physiopathologie de Toulouse Purpan, Paul Sabatier University, Toulouse, France,Caroline.charlier@inserm.fr
Chelbi-Alix,Mounira K	CNRS FRE3235, Université Paris Descartes, Paris, France,mounira.chelbi-alix@parisdescartes.fr
Cheng,Benson	University of Rochester,benson_cheng@urmc.rochester.edu
Chepurnov,Alexander	Institute of Clinical immunology,alexa.che.purnov@gmail.com
Chinikar,Sadegh	Arboviruses and Viral Haemorrhagic Fevers Laboratory (National Ref. Lab), Department of Virology, Pasteur Institute of Iran,Sadeghchinikar@yahoo.com
Conzelmann,Karl-Klaus	LMU Munich,conzelma@lmb.uni-muenchen.de
Cosby,Louise	Queen's University Belfast,l.cosby@qub.ac.uk
Cusi,Maria Grazia	University of Siena,mariagrazia.cusi@unisi.it
Dacheux,Laurent	Institut Pasteur,laurent.dacheux@pasteur.fr
Davey,Robert	Texas Biomedical Research Institute,rdavey@txbiomed.org
de Graaf,Miranda	Department of Viroscience, Erasmus Medical center,m.degraaf@erasmusmc.nl
de Haan,C.A.M. (Xander)	Virology Division, Utrecht University,c.a.m.dehaan@uu.nl
de Prat Gay,Gonzalo	Fundación Instituto Leloir. - IIBBA CONICET,gpg@leloir.org.ar
De Swart,Rik	Erasmus MC, department Viroscience,r.deswart@erasmusmc.nl
De Vito,Ilaria	Weill Cornell Medical College,ilariadevito@gmail.com
de Wit,Emmie	National Instiues of Health,Emmie.deWit@nih.gov
Debra,Debra	Animal Health Trust,debra.elton@aht.org.uk
DeBuysscher,Blair	National Institutes of Health,debuysscherb@niaid.nih.gov
Deng,Guohua	Harbin Veterinary Research Institute of CAAS, P. R. China,dgh@hvri.ac.cn
Deng,Tao	Institute of Pathogen Biology, Chinese Academy of Medical Sciences,dtapple@yahoo.com
Dibben,Oliver	Icahn School of Medicine at Mount Sinai,oliver.dibben@mssm.edu
Dietzel,Erik	Institute of Virology, Philipps-University Marburg,erik.dietzel@staff.uni-marburg.de
DiNapoli,Joshua	Sanofi Pasteur,joshua.dinapoli@sanofipasteur.com
Dodd,Kimberly	US Centers for Disease Control and Prevention,isx8@cdc.gov

DOI,TOMOMITSU	International research Center for Infectious Diseases, Institute of Medical Science, University of Tokyo,doitomo@ims.u-tokyo.ac.jp
Duprex,Paul	Boston University School of Medicine,pduprex@bu.edu
Edinger,Thomas	University of Zurich, Switzerland,edinger.thomas@virology.uzh.ch
Eggink,Dirk	Mount Sinai School of Medicine,dirk.eggink@mssm.edu
Eisfeld,Amie	University of Wisconsin-Madison,aefenney@vetmed.wisc.edu
El Najjar,Farah	Department of Molecular and Cellular Biochemistry, University of Kentucky,farah.najjar@uky.edu
ELEOUËT,Jean-François	INRA,jean-francois.eleouet@jouy.inra.fr
Elliott,Richard	Univerity of Glasgow,richard.elliott@glasgow.ac.uk
Escriou,Nicola	Institut Pasteur, Paris, France,nicolas.escriou@pasteur.fr
ESCUDERO PEREZ,Beatriz	International Centre for Research in Infectiology (CIRI), INSERM U1111/ CNRS UMR5308 - Molecular Basis of Viral Pathogenicity,beatriz.escudero-perez@inserm.fr
Esperante,Sebastián	Fundación Instituto Leloir. - IIBBA CONICET.,sesperante@leloir.org.ar
ESSAIDI-LAZIOSI,Manel	Mrs.,manel.essaidi@unige.ch
Evander,Magnus	Department of Clinical Microbiology, Division of Virology, Umeå University,-magnus.evander@climi.umu.se
Evans,Jennifer	University of Warwick/AHVLA,jennifer.s.evans@warwick.ac.uk
Falcon,Ana	National Center of Biotechnology (CNB-CSIC),afalcon@cnb.csic.es
Feagins,Alicia	Icahn School of Medicne at Mount Sinai,alicia.feagins@mssm.edu
Fearns,Rachel	Boston University School of Medicine,rfearns@bu.edu
Fehling,Sarah Katharina	Institute of Virology, Philipps-University Marburg, Germany,fehling@staff.uni-marburg.de
Feinman,Leighland	Icahn School of Medicine at Mount Sinai,leighland.feinman@mssm.edu
Fernandez-Muñoz,Rafael	Unidad de Virología del Hospital Ramon y Cajal, rafael.fernandez-mu@gmail.com
Fernandez,Manolo	FARVET SAC,farvet@farvet.com
Finke,Stefan	Institute of Molecular Biology, Friedrich-Loeffler-Institut, stefan.finke@fli.bund.de
Flint,Michael	Centers for Disease Control and Prevention,vfa3@cdc.gov
Fodor,Ervin	University of Oxford,ervin.fodor@path.ox.ac.uk
Fonseca Aguilar,Wendy Fabiola	Centro de Investigación y Estudios Avanzados del I.P.N., wfonseca.ag@gmail.com
Freiberg,Alexander	University of Texas Medical Branch,anfreibe@utmb.edu
Frensing,Timo	Max Planck Institute for Dynamics of Complex Technical Systems, frensing@mpi-magdeburg.mpg.de
Fu,Zhen	University of Georgia,zhenfu@uga.edu
Fu,Zhen	University of Georgia,zhenfu@uga.edu
Fujino,Kan	Department of Viral Oncology, Institute for Virus Research, Kyoto University fujino@biken.osaka-u.ac.jp
Fuller,Chad	AHVLA - Weybridge,chad.fuller@ahvla.gsi.gov.uk
Gabriel,Gülsah	Heinrich-Pette-Institute, Leibniz Institute for Experimental Virology,guelsah.gabriel@hpi.uni-hamburg.de
GALLOUX,Marie	INRA,marie.galloux@jouy.inra.fr
Galloway,Summer	Emory University School of Medicine, Atlanta, GA, USA,sgallo2@emory.edu
Garcia-Sastre,Adolfo	Icahn School of Medicine at Mount Sinai,adolfo.garcia-sastre@mssm.edu

Garcin,Dominique	MIMO Department,dominique.garcin@unige.ch
Garten,Rebecca	Centers for Disease Control/Influenza Division,dqy5@cdc.gov
Gaudin,Yves	CNRS,yves.gaudin@vms.cnrs-gif.fr
Ge,Jinying	Harbin Veterinary Research Institute,Chinese Academy of Agricultural Sciences gjy2003@163.com
Gerlach,Piotr	EMBL Grenoble,pgerlach@embl.fr
Gerlier,Denis	CIRI, INSERM U1111, CNRSUMR5308, UCBL, ENS Lyon, denis.gerlier@inserm.fr
Ghanem,Alexander	LMU Munich,ghanem@lmb.uni-muenchen.de
Ghildyal,Reena	University of Canberra,Reena.Ghildyal@canberra.edu.au
Glennon,Nicole	Icahn School,nicole.glennon@mssm.edu
Gómez,Beatriz	Universidad Nacional Autónoma de México,begomezq@unam.mx
Gonzalez-Dunia,Daniel	INSERM U1043, Centre de Physiopathologie de Toulouse Purpan, Paul Sabatier University, Toulouse, France,daniel.dunia@inserm.fr
Gorman,Jeffrey	Queensland Institute of Medical Research (QIMR),jeff.gorman@qimr.edu.au
Griffiths,Anthony	Texas Biomedical Research Instittue,agriffiths@txbiomed.org
Groseth,Allison	National Institutes of Health,allison_groseth@nih.gov
Guerra,Jessica	Department of Microbiology and Molecular Medicine, CMU University of Geneva,jessica.guerra@unige.ch
Gupta,Shawon	Karolinska Institutet,shawon.gupta@ki.se
Gutsche,Irina	Unit of Virus Host Cell Interactions,gutsche@embl.fr
Haid,Sibylle	TWINCORE,sibylle.haid@twincore.de
haijema,bertjan	Mucosis ,bertjan.haijema@mucosis.com
Hale,Benjamin	University of Glasgow,ben.hale@glasgow.ac.uk
Haller,Otto	Department of Virology, University of Freiburg, Germany, otto.haller@uniklinik-freiburg.de
Halmann,Peter	University of Wisconsin-Madison,halfmanp@vetmed.wisc.edu
Hangartner,Lars	University of Zurich,hangartner.lars@virology.uzh.ch
Hara,Koyu	Kurume University School of Medicine,koyu@med.kurume-u.ac.jp
Hartman,Amy	University of Pittsburgh ,hartman2@pitt.edu
Hatta,Masato	University of Wisconsin-Madison,vgroth@svm.vetmed.wis.edu
He,Biao	University of Georgia,bhe@uga.edu
Herrler Georg	Institute of Virology, University of Veterinary Medicine Hannover, Germany,-Georg.Herrler@tiho-hannover.de
Hoenen,Thomas	National Institutes of Health,thomas_hoenen@nih.gov
Höfs,Nadja	Zymo Research Europe,nhoefs@zymoresearch.de
Holzer,Barbara	The Pirbright Institute,barbara.holzer@pirbright.ac.uk
Honda,Ayae	Department of Frontier Bioscience, Hosei University,ayhonda@hosei.ac.jp
Honda,Tomoyuki	Department of Viral Oncology, Institute for Virus Research, Kyoto University,-thonda@virus.kyoto-u.ac.jp
Horvat,Branka	CIRI, INSERM,branka.horvat@inserm.fr
Igarashi,Manabu	Hokkaido University,igarashi@czc.hokudai.ac.jp
Ilyushina,Natalia	Geisel Medical School at Dartmouth,Natalia.A.Ilyushina@Dartmouth.Edu
Irie,Takashi	Department of Virology, Institute of Biomedical & Health Sciences, Hiroshima University,tirie@hiroshima-u.ac.jp
Ito,Naoto	Faculty of Applied Biological Sciences, Gifu University,naotoito@gifu-u.ac.jp

Iwatsuki-Horimoto,Kiyoko	Division of Virology, Institute of Medical Science, University of Tokyo,kenken@ims.u-tokyo.ac.jp
Jamin,Marc	UMI3265 Unit of Virus Host Cell Interactions,jamin@embl.fr
Jiang,Yongping	Harbin Veterinary Research Institute, CAAS,ypjiang99@163.com
Jin,Hong	MedImmune,jinh@medimmune.com
Johnson,Patricia	Dublin City University,patricia.johnson@dcu.ie
Kai,Chieko	The University of Tokyo,ckai@ims.u-tokyo.ac.jp
Kainulainen,Markus	Philipps-University Marburg,markus.kainulainen@staff.uni-marburg.de
Kajihara,Masahiro	Hokkaido University Research Center for Zoonosis Control, kajihara@czc.hokudai.ac.jp
Kariwa,Hiroaki	Hokkaido University,kariwa@vetmed.hokudai.ac.jp
Kashiwagi,Takahito	Kurume University School of Medicine,takahito@med.kurume-u.ac.jp
Katsura,Hiroaki	Division of Virology, Institute of Medical Science University of Tokyo, kk096367@mgs.k.u-tokyo.ac.jp
Kaverin,Nikolai	D.I. Ivanovsky Instiitute of Virology, labphysvir@mail.ru
Kawaguchi,Atsushi	Department of Infection Biology, Faculty of Medicine, University of Tsukuba, ats-kawaguchi@md.tsukuba.ac.jp
Kawaoka,Yoshihiro	University of Wisconsin-Madison, kawaokay@svm.vetmed.wisc.edu
Keita,Matsuno	National Institutes of Health,keita.matsuno@nih.gov
Killip,Marian	University of St Andrews,mjk3@st-andrews.ac.uk
Kindsmüller,Kathrin	Emergent Product Development Germany GmbH, kindsmuellerk@ebsi.com
Klaus,Joseph	University of Vermont,jklaus@uvm.edu
Klingström,Jonas	Department of Preparedness, Swedish Institute for Communicable Diseases Control, SE-171 82 Solna, Sweden, jonas.klingstrom@smi.se
Knepper,Jessica	Robert Koch Institute, Division 17 Influenza and Other Respiratory Viruses, Berlin,KnepperJ@rki.de
Kochs,Georg	University of Freiburg,georg.kochs@uniklinik-freiburg.de
Kolakofsky,Daniel	Dept Microbiology, University Geneva Medical School,kolakofsky@yahoo.com
Kortekaas,Jeroen	Central Veterinary Institute of Wageningen University and Research Centre, jeroen.kortekaas@wur.nl
Krafft,Amy	National Institute for Allergy and Infectious Diseases, NIH, kraffta@niaid.nih.gov
Kreher,Felix	Institut Pasteur, fkreher@pasteur.fr
Krumm,Stefanie	Georgia State University, skrumm@emory.edu
Krzyzaniak,Magdalena	ETH Zurich, magdalena.krzyzaniak@bc.biol.ethz.ch
Kuhen,Kelli,	Novartis - GNF, kkuhen@gnf.org
Kuhn,Jens H.	NIH/NIAID Integrated Research Facility at Fort Detrick, kuhnjens@mail.nih.gov
Kunz,Stefan	Institute of Microbiology University of Lausanne, Stefan.Kunz@chuv.ch
Kurosaki,Yohei	Institute of Tropical Medicine, Nagasaki University,ykuro@nagasaki-u.ac.jp
Kurosaki,Yohei	Department of Emerging Infectious Diseases, Institute of Tropical Medicine, Nagasaki University,ykuro@nagasaki-u.ac.jp
Kwon,Hyunjeong	Laboratory Animal Research Center, Institute of Medical Science, The University of Tokyo., eboshipv@ims.u-tokyo.ac.jp
LAGAUDRIERE,CECILE	CNRS, cecile.lagaudriere@vms.cnrs-gif.fr
Lage Ferreira,Helena	FZEA-USP, hlage@usp.br

Lamb,Robert	HHMI and Northwestern University, ralamb@northwestern.edu
Lamp,Boris	Institute of Virology, Philipps-University Marburg, boris.lamp@staff.uni-marburg.de
Landeras Bueno,Sara	Centro Nacional de Biotecnologia, slanderas@cnb.csic.es
Larrous,Florence	Institut Pasteur, Unit of Lyssavirus Dynamics and Host Adaptation, florence.larrous@pasteur.fr
Lasecka,Lidia	The Pirbright Institute,lidia.lasecka@pirbright.ac.uk
Last,First	University, person@yahoo.com
Lawrence,Philip	International Centre for Research in Infectiology (CIRI), INSERM U1111/CNRS UMR5308 - Molecular Basis of Viral Pathogenicity, philip.lawrence@inserm.fr
LE GOFFIC,Ronan	INRA, ronan.legoffic@jouy.inra.fr
Le Nouën,Cyril	NIH/NIAID/LID, lenouenc@niaid.nih.gov
Lee,Benhur	David Geffen School of Medicine at UCLA, bleebhl@ucla.edu
Lehmann,Maria	Bernhard-Nocht-Institute for Tropical Medicine, maria.lehmann@hotmail.de
Levingston Mac leod,Jesica	Mount Sinai School of Medicine, jesica.levingstonmacleod@mssm.edu
Leyrer,Sonja	Emergent Product Development Germany GmbH, erdeljii@ebsi.com
LI,CHENGJUN,	Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences (CAAS), LICHENGJUN@CAAS.CN
Liang,Mifang	National Institute for Viral Disease Control and Prevention, China CDC, mifangl@vip.sina.com
Liang,Xiaoping	Merck & Co. Inc., xiaoping.liang@merck.com
Liang,Yuhong	Icahn school of medicine at mount sinai, Yuhong.liang@mssm.edu
Liang,Yuying	University of Minnesota, liangy@umn.edu
Liu,Jinxiong	Harbin veterinary institute of CAAS, P.R China, hero7983@yahoo.com.cn
Lo,Michael	Centers for Disease Control and Prevention, Viral Special Pathogens Branch, mko2@cdc.gov
Lo,Michael	Centers for Disease Control and Prevention, Viral Special Pathogens Branch, mko2@cdc.gov
Longhi,Sonia	AFMB, UMR 7257, CNRS and Aix-Marseille Université,Sonia. Longhi@afmb.univ-mrs.fr
Lopez,Nora	CEVAN-ICT Milstein, CONICET, nlopezcevan@centromilstein.org.ar
LOUBER,Jade	INSERM - CIRI U1111, jade.louber@inserm.fr
Lowen,Anice	Emory University, anice.lowen@emory.edu
Ludlow,Martin	Boston University, mludlow@bu.edu
Ludwig,Stephan	Institute of Molecular Virology - University of Muenster, ludwigs@uni-muenster.de
Luo,Ming	University of Alabama at Birmingham, mingluo@uab.edu
Luthra,Priya	Mount SInai School of Medicine, priya.luthra@mssm.edu
Ly,Hinh	University of Minnesota, hly@umn.edu
Maisner,Andrea	Institute of Virology, Marburg, maisner@staff.uni-marburg.de
Makino,Akiko	Kyoto University, amakino@virus.kyoto-u.ac.jp
Mani,Nagraj	Vertex Pharmaceuticals Inc, Nagraj_Mani@vrtx.com
Mänz,Benjamin	Erasmus MC Rotterdam, b.manz@erasmusmc.nl
Manzoor,Rashid	Division of Global Epidemiology, Hokkaido University Research Center for Zoonosis Control, Japan, manzoor@czc.hokudai.ac.jp
MARC,Daniel	Institut National de la Rercherche Agronomique,daniel.marc@tours.inra.fr

Marcos Villar,Laura	CENTRO NACIONAL DE BIOTECNOLOGIA, lmarcos@cnb.csic.es
Markowska-Daniel,Iwona	The National Veterinary Research Institute, Pulawy, Poland, iwonamd@piwet.pulawy.pl
Marsh,Glenn	CSIRO Australian Animal Health Laboratory, glenn.marsh@csiro.au
Martínez Alonso,Mónica	University of Oxford, monica.martinezalonso@path.ox.ac.uk
Martinez-Romero,Carles	Icahn School of Medicine at Mount Sinai, carles.martinez@mssm.edu
Martinez-Sobrido,Luis	University of Rochester, luis_martinez@urmc.rochester.edu
Martinez-Sobrido,Luis	University of Rochester,luis_martinez@urmc.rochester.edu
Mas,Vicente	Centro Nacional Microbiología_Instituto de Salud Carlos III, vmas@isciii.es
MATEO,Mathieu	Mayo Clinic,mateo.mathieu@mayo.edu
MATHIEU,Cyrille	Weill Cornell Medical College, cyrille.mathieu69@gmail.com
McElroy,Anita	Centers for Disease Control and Prevention,gsz5@cdc.gov
McElroy,Anita	US Centers for Disease Control and Prevention, gsz5@cdc.gov
McKimm-Breschkin,Jenny	CSIRO Materials Science and Engineering, mck245@csiro.au
McQuaid,Samantha	Dublin City University, samantha.mcquaid2@gmail.com
MELERO,JOSÉ A.	CENTRO NACIONAL DE MICROBIOLOGÍA, jmelero@isciii.es
Mena,Ignacio	Icahn School of Medicine at Mount Sinai. New York (NY) USA, mena.nacho@mssm.edu
Mettenleiter,Thomas	Friedrich-Loeffler-Institut, thomas.mettenleiter@fli.bund.de
Misasi,John	Boston Children's Hospital, john.misasi@chboston.org
Mohan,Gopi	Emory University, gmohan@emory.edu
Momose,Fumitaka	Kitasato Institute for Life Sciences, Kitasato University, fmomose@lisci.kitasato-u.ac.jp
Morin,Benjamin	Harvard Medical School, Benjamin_Morin@hms.harvard.edu
Moscona,Anne	Weill Cornell Medical College,anm2047@med.cornell.edu
Moseley,Gregory	Monash, greg.moseley@monash.edu
Mottet-Osman,Geneviève	Department of Microbiology and Molecular Medicine, CMU University of Geneva, genevieve.mottet@unige.ch
Motz,Carina	Gene Center Munich, LMU, motz@genzentrum.lmu.de
Mühlberger,Elke	Boston University School of Medicine, Dept. Microbiology, muehlber@bu.edu
Munir,Shirin	National Institutes of Health, munirs@niaid.nih.gov
Muniraju,Murali	The Pirbright Institute, murali.muniraju@pirbright.ac.uk
Munoz-Fontela,Cesar	Heinrich Pette Institute, Leibniz Institute For Experimental Virology, cesar.munoz-fontela@hpi.uni-hamburg.de
Muñoz Alía,Miguel Ángel	Unidad de Virología, Hospital Ramón y Cajal, Madrid, Spain, miguelangel.munoz@salud.madrid.org
Muraki,Yasushi	Department of Microbiology, Kanazawa Medical University School of Medicine, ymuraki@kanazawa-med.ac.jp
Nakamura,Shoko	Department of Viral Oncology, Institute for Virus Research, Kyoto University, Japan, nakamura.shoko.87c@st.kyoto-u.ac.jp
Nash,Bethany	AHVLA, bethany.nash@ahvla.gsi.gov.uk
Naslund,Jonas	FOI, Swedish Defence Research Agency, jonas.naslund@foi.se
Nemirov,Kirill	Molecular basis of high pathogenicity of viral infections, INSERM CIRI U1111,-kirill.nemirov@inserm.fr
Neumann,Gabriele	University of Wisconsin-Madison, neumanng@svm.vetmed.wisc.edu
Newcomb,Laura	California State University San Bernardino, lnewcomb@csusb.edu

Nichol,Stuart Centers For Disease Control,stn1@cdc.gov

Nieto,Amelia Centro Nacional de Biotecnologia.CSIC, anieto@cnb.csic.es

NIKOLIC,Jovan LVMS - CNRS UPR 3296, nikolic@vms.cnrs-gif.fr

Nilsson,Elin FOI, Swedish Defence Research Agency, elin.nilsson@foi.se

Nishio,Machiko Wakayama Medical University, mnishio@wakayama-med.ac.jp

Noda,Takeshi University of Tokyo,t-noda@ims.u-tokyo.ac.jp

Nolden,Tobias Friedrich-Loeffler-Institute, tobias.nolden@fli.bund.de

Noton,Sarah Boston University School of Medicine, slnoton@bu.edu

Nyanguile,Origene University of Applied Sciences Western Switzerland, origene.nyanguile@hevs.ch

Ogino,Tomoaki Cleveland Clinic, oginot@ccf.org

Ohkura,Takashi Department of Infection Biology, Division of Biomedical Science, Faculty of Medicine, University of Tsukuba, t-ohkura@md.tsukuba.ac.jp

Ohta,Keisuke Department of Microbiology, Wakayama Medical University, Japan, k-ooota@wakayama-med.ac.jp

Olivier,LEYMARIE INRA, olivier.leymarie@jouy.inra.fr

Oreshkova,Nadia Central Veterinary Institute of Wageningen UR (CVI-Lelystad), nadia.oreshkova@wur.nl

ORTIN,JUAN CENTRO NACIONAL DE BIOTECNOLOGIA (CSIC), jortin@cnb.csic.es

Ortiz-Riaño,Emilio University of Rochester, eortizriano@urmc.rochester.edu

Otsuki,Noriyuki Department of Virology3, National Institute of Infectious Diseases, otsuki@nih.go.jp

Otte,Anna Heinrich Pette Institute, Leibniz Institute for Experimental Virology , anna.otte@hpi.uni-hamburg.de

Ouizougun-Oubari,Mohamed Institut Pasteur,mouizoug@pasteur.fr

Ozawa,Makoto Laboratory of Animal Hygiene, Joint Faculty of Veterinary Medicine, Kagoshima University, mozawa@vet.kagoshima-u.ac.jp

Palmarini,Massimo MRC-University of Glasgow Centre for Virus Research, massimo.palmarini@glasgow.ac.uk

PAPA,ANNA Professor in Microbiology, annap@med.auth.gr

Parida,Satya Prof, satya.parida@pirbright.ac.uk

Park,Arnold UCLA, arnold.park@gmail.com

Parrington,Mark Sanofi Pasteur, mark.parrington@sanofipasteur.com

Pasquato,Antonella Institute of Microbiology (IMUL); University Hospital Center and University of Lausanne (CHUV), antonella.pasquato@chuv.ch

Paterson,Duncan University of Oxford, duncan.paterson@path.ox.ac.uk

Paterson,Reay Northwestern University, r-paterson@northwestern.edu

Patterson,Jean Texas Biomedical Research Institute, jpatters@txbiomed.org

Patzina,Corinna Institute for Virology, University of Freiburg, corinna.patzina@uniklinik-freiburg.de

Peitsch,Catharina Institute of Virology, Philipps University Marburg, Hans-Meerwein-Straße 2, 35043 Marburg, Germany, catharina.peitsch@gmx.de

Pentecost,Mickey University of California Los Angeles,mickeypentecost@ucla.edu

Perez Giron,Jose Vicente Heinirich Pette Institut, joeviperez@gmail.com

Pérez-Cidoncha,Maite Centro Nacional de Biotecnología, CSIC / Ciber de Enfermedades Respiratorias, ISCIII. , tperez@cnb.csic.es

Pernet,Olivier University of Californial, Los Angeles,opernet@ucla.edu

Pietschmann,Thomas	TWINCORE, thomas.pietschmann@twincore.de
Plattet,Philippe	Vetuisse Faculty, University of Bern, philippe.plattet@vetsuisse.unibe.ch
Plemper,Richard	Georgia State University, rplempe@emory.edu
Pohl,Marie-Theres	Institute of Medical Virology, University of Zurich, pohl.marie-theres@virology.uzh.ch
Power,Ultan	Queen's University Belfast, u.power@qub.ac.uk
Prescott,Joseph	National Insitutes of Health, prescottjb@niaid.nih.gov
Pretel,Esteban	Fundación Instituto Leloir. - IIBBA CONICET, epretel@leloir.org.ar
Pyo,Hyun Mi	Vaccine and Infectious Disease Organization, University of Saskat-chewan,ph7926@gmail.com
Raaben,Matthijs	Harvard Medical School, matthijs_raaben@hms.harvard.edu
Radoshitzky,Sheli	USAMRIID, sheliijo@gmail.com
Rajsbaum,Ricardo	Department of Microbiology, Icahn School of Medicine at Mount Sinai, ricardo.rajsbaum@mssm.edu
RAMEIX-WELTI,Marie-Anne	Université de Versailles St-Quentin - Epidémiologie et Physiolopathologie des Infections Microbiennes, ma.welti@free.fr
Randall,Richard	University of St Andrews, rer@st-andrews.ac.uk
Ranjan,Punya	Centers for Disease Control and Prevention ,ifh0@cdc.gov
Ranjan,Punya,	Viral Special Pathogens Branch, DHCPP, NCEZID, OID, CDC, pranjan1@cdc.gov
Rash,Adam	Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk, CB8 7UU, UK,
Reed,Douglas	University of Pittsburgh, dsreed@pitt.edu
Reguera,Juan	EMBL Grenoble, jreguera@embl.fr
Rennick,Linda	Boston University, rennick@bu.edu
Resa-Infante,Patricia	Heinrich-Pette-Institute, Leibniz Institute for Experimental Virology, patriciaresainfante@gmail.com
Reynard,Frederic,	MERIAL, frederic.reynard@merial.com
Riblett,Amber,	University of Pennsylvania,amber.riblett@gmail.com
Richardson,Christopher	Dalhousie University,chris.richardson@dal.ca
Richt,Juergen	Kansas State University Veterinary College,akb6666@vet.k-state.edu
Rieger,Toni,	Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany, rieger@bni-hamburg.de
Rima,Bert	Queen's University Belfast ,b.rima@qub.ac.uk
Robinson,Lindsey	Department of Microbiology and Immunobiology, Harvard Medical School,
Robinson,Lindsey	Harvard Medical School,lrobins@fas.harvard.edu
Römer-Oberdörfer,Angela	Friedrich-Loeffler-Institut,angela.roemer-oberrdoerfer@fli.bund.de
Rossman,Jeremy	University of Kent,j.s.rossman@kent.ac.uk
Ruigrok,Rob	Université Joseph Fourier, Grenoble, France,ruigrok@embl.fr
Russell,Charles	St. Jude Children's Research Hospital, Memphis, TN, U.S.A., charles.russell@stjude.org
RUSSIER,Marion	INSTITUT PASTEUR - UBIVE, marion.russier@inserm.fr
Saelens,Xavier	Ghent University and VIB,xavier.saelens@dmbr.vib-ugent.be
Saffarian,Saveez	University of Utah,saffarian@physics.utah.edu
SAITO,Takehiko	National Institute of Animal Health,taksaito@affrc.go.jp
Sakai,Kouji	National Institute of Infectious Diseases,ksakai@nih.go.jp

Sakurai,Yasuteru	Texas Biomedcal Research Institute,ysakurai@txbiomed.org
salvatore,mirella	Weill Medical College of Cornell University,mis2053@med.cornell.edu
SANCHEZ-APARICIO,MARIA	ICAHN SCHOOL OF MEDICINE AT MOUNT SINAI, maria.t.sanchez@mssm.edu
Sanz Bernardo,Beatriz	The Pirbright Institute,beatriz.sanz-bernardo@pirbright.ac.uk
Sato,Hiroki	Laboratory Animal Research Center, Institute of Medical Science, The University of Tokyo, satohi@ims.u-tokyo.ac.jp
Sauerhering,Lucie	Institute of Virology, Philipps-University Marburg, Marburg, Germany, sauerhel@staff.uni-marburg.de
Schierhorn,Kristina L.	Robert Koch-Institute, Influenza and respiratory Viruses,schierhornk@rki.de
Schmidt,Kristina	Boston University,schmidtk@bu.edu
Schmolke,Mirco	Icahn School of Medicine at Mount Sinai,mirco.schmolke@mssm.edu
Schmolke,Mirco	Icahn School of Medicine at Mount Sinai,mirco.schmolke@mssm.edu
Schudt,Gordian	Institute of Virology - Philipps-University of Marburg, schudtg@staff.uni-marburg.de
Schwemmle,Martin	Deaprtment of Virology, University of Freiburg, martin.schwemmle@uniklinik-freiburg.de
Sedeyn,Koen	Department for Molecular Biomedical Research, VIB, Ghent University, Technologiepark 927, Ghent, Belgium,koen.sedeyn@dmbr.vib-UGent.be
Seregin,Alexey	University of Texas Medical Branch, Galveston, USA,alseregi@utmb.edu
Shaw,Megan	Icahn School of Medicine at Mount Sinai,megan.shaw@mssm.edu
Shevtsova,Anastasia	University of Geneva,anastasia.shevtsova@unige.ch
Shimizu,Kenta	Hokkaido University Graduate School of Medicine, kshimizu@med.hokudai.ac.jp
Shimojima,Masayuki	National Institute of Infectious Diseases ,shimoji-@nih.go.jp
Shtanko,Olena	Texas Biomedical Research Institute,oshtanko@txbiomed.org
Silaghi,Alex	University of Manitoba,alex_silaghi@hotmail.com
Sizun,Christina	Institut de Chimie des Substances Naturelles CNRS UPR2301, sizun@icsn.cnrs-gif.fr
Sleeman,Katrina	Centers for Disease Control and Prevention, Influenza Division, KSleeman1@cdc.gov
Sleeman,Katrina	CDC,hhk6@cdc.gov
Soda,Kosuke	Department of Veterinary Medicine, Faculty of Agriculture, Tottori University,-soda@muses.tottori-u.ac.jp
SOURIMANT,Julien	INRA,julien.sourimant@jouy.inra.fr
Sparrer,Konstantin,	LMU Munich,sparrer@lmb.uni-muenchen.de
Spiropoulou,Christina	Centers for Disease Control and Prevention,ccs8@cdc.gov
Spiropoulou,Christina,	Centers for Disease Control and Prevention,ccs8@cdc.gov
Staeheli,Peter	University of Freiburg,peter.staeheli@uniklinik-freiburg.de
Stech,Juergen,	Friedrich-Loeffler-Institut,juergen.stech@fli.bund.de
Steel,John,	Emory University,john.steel@emory.edu
Steglich,Constanze	Friedrich-Loeffler-Institut,Constanze.Steglich@fli.bund.de
Stephane,DUQUERROY	Pasteur Institute,sduquer@pasteur.fr
Stertz,Silke	University of Zurich,stertz.silke@virology.uzh.ch
Stevaert,Annelies	Rega Institute for Medical Research, KU Leuven, annelies.stevaert@rega.kuleuven.be

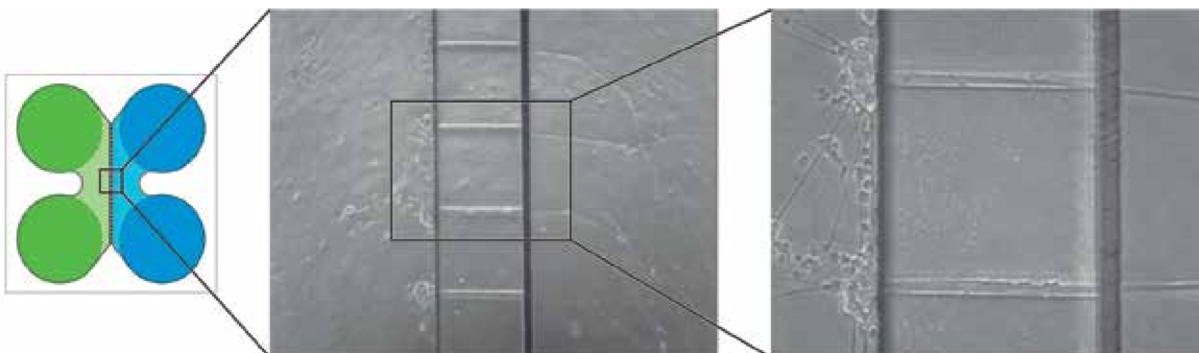
Strecker,Thomas	Institute of Virology, Philipps-University Marburg, Germany, strecker@staff.uni-marburg
Stroeher,Ute	Centers for Disease Control,ixy8@cdc.gov
Ströher,Ute	CDC, Viral Special Pathogens Branch,ixy8@cdc.gov
Subbiah,Elankumaran	Virginia Polytechnic Institute and STate University,kumarans@vt.edu
Sugai,Akihiro	The Institute of Medical Science, The University of Tokyo, sugai@ims.u-tokyo.ac.jp
Sugita,Yukihiko	Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo,y-sugita@ims.u-tokyo.ac.jp
Szelechowski,Marion	INSERM U1043, Centre de Physiopathologie de Toulouse Purpan, Paul Sabatier University, Toulouse, France,marion.szelechowski@inserm.fr
Tahara,Maino	National Institute of Infectious Diseases,maino@nih.go.jp
Takada,Ayato	Hokkaido University Research Center for Zoonosis Control, atakada@czc.hokudai.ac.jp
Takashita,Emi	National Institute of Infectious Diseases,emitaka@nih.go.jp
Takeda,Makoto	National Institute of Infectious Diseases,mtakeda@nih.go.jp
Takimoto,Toru	University of Rochester Medical Center,toru_takimoto@urmc.rochester.edu
Tani,Hideki	National Institute of Infectious Diseases,htani@nih.go.jp
Tao,Yizhi Jane	Rice University,ytao@rice.edu
Thorsten,Wolff	Robert Koch-Institut,WolffT@RKI.de
Thulasi Raman,Sathya Narayanan	VIDO-InterVac (University of Saskatchewan, Canada), chatyabiotech@gmail.com
Tischler,Nicole	Molecular Virology Laboratory, Fundación Ciencia & Vida, ntischler@cienciavida.cl
Tomonaga,Keizo	Institute for Virus Research, Kyoto University,tomonaga@virus.kyoto-u.ac.jp
Tompkins,Mark	University of Georgia,smt@uga.edu
Tong,Suxiang	Division of Viral Diseases, CDC,sot1@cdc.gov
Towner,Jonathan	Centers for Disease Control and Prevention,jit8@cdc.gov
Tripp,Ralph	University of Georgia,ratripp@uga.edu
Tsuda,Yoshimi	Hokkaido University, Graduate School of Medicine,tsuday@med.hokudai.ac.jp
Tsunekuni,Ryota	National Institute of Animal Health, National Agriculture and Food Research Organization,tune@affrc.go.jp
Tsunekuni,Ryota	National Institute of Animal Health, National Agriculture and Food Research Organization,tune@affrc.go.jp
Tsurudome,Masato	Mie University Graduate School of Medicine,turudome@doc.medic.mie-u.ac.jp
Turrell,Lauren	Oxford University,lauren.turrell@path.ox.ac.uk
Uchida,Yuko	National Institute of Animal Health,uchiyu@affrc.go.jp
Uebelhoer,Luke	Centers for Disease Control and Prevention,vsp0@cdc.gov
Ulrich,Rainer G.	Friedrich-Loeffler-Institut,rainer.ulrich@fli.bund.de
Uraki,Ryuta	Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo,kk106364@mgs.k.u-tokyo.ac.jp
Urata,Shuzo	Nagasaki University, Institute of Tropical Medicine,shuzourata@nagasaki-u.ac.jp
Vakharia,Vikram	University of Maryland Baltimore County,vakharia@umbc.edu
Van den Hoecke,Silvie	VIB Department for Molecular Biomedical Research, UGent, silvie.vandenhoecke@dmbr.ugent.be
Vanderlinden,Evelien	Rega Institute for Medical Research,evelien.vanderlinden@rega.kuleuven.be

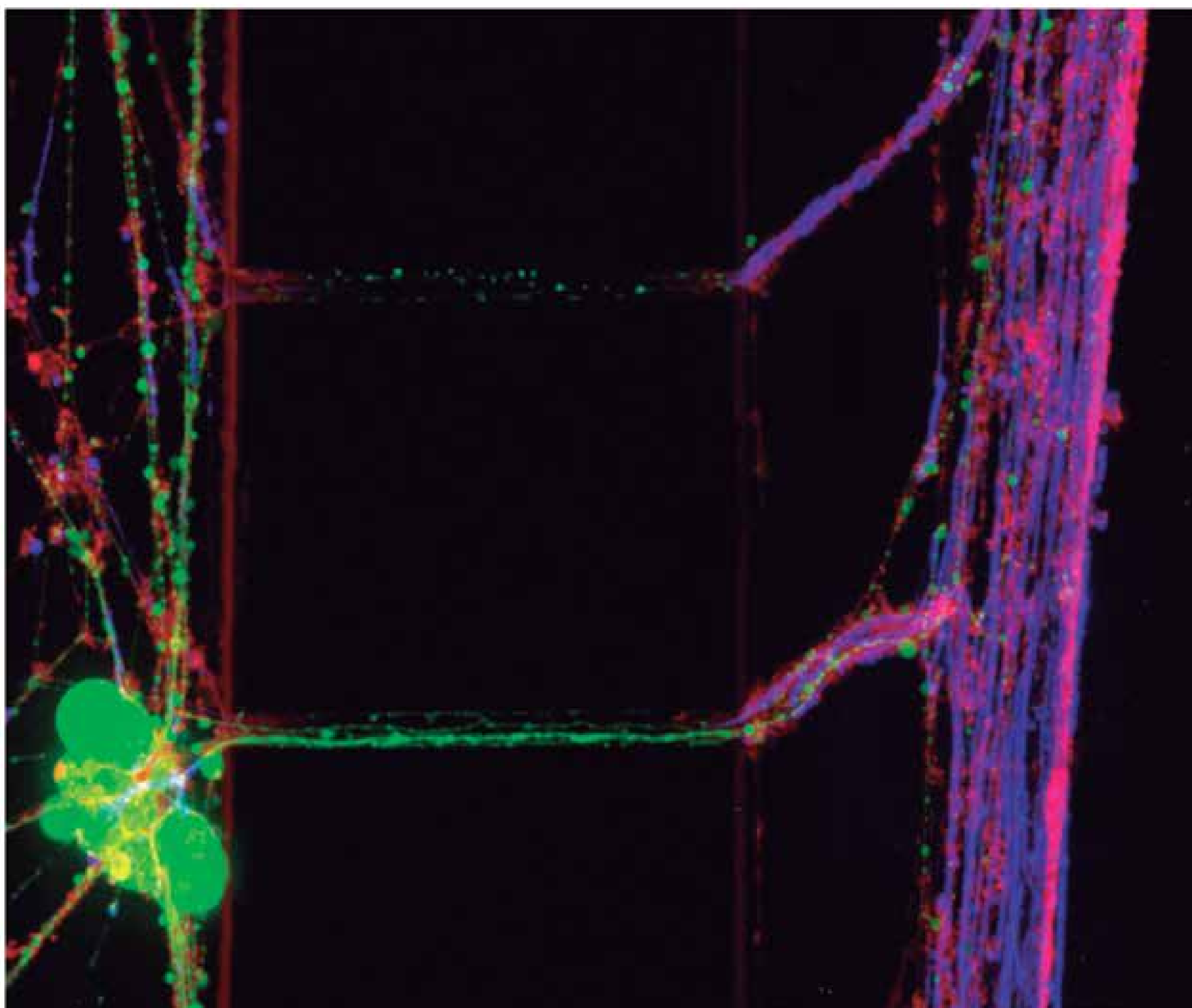
Verhelst,Judith	VIB-DMBR, Ghent University, Belgium,judith.verhelst@dmbr.vib-ugent.be
Viegas,Mariana	Virology Laboratory, Dr. Ricardo Gutiérrez Children's Hospital, Buenos Aires, Argentina.,viegasmariana@hotmail.com
Vijayakrishnan,Swetha	MRC-University of Glasgow Centre for Virus Research, Swetha. Vijayakrishnan@glasgow.ac.uk
Villanueva ,Nieves	Cantro Nacional de Microbiologia (CNM).Instituto de Salud Carlos III (ISCIII), nvilla@isciii.es
Volchkov,Viktor	Molecular basis of viral pathogenicity, CIRI, INSERM U1111, Université de Lyon,viktor.volchkov@inserm.fr
Volchkova,Valentina	CIRI, INSERM U1111, Université Lyon 1, Molecular Basis of Viral Pathogenicity, valentina.volchkova@inserm.fr
Vreede,Frank	University of Oxford, frank.vreede@path.ox.ac.uk
Vries,Erik	Virology Division, Faculty of Veterinary Medicine, Utrecht University, e.devries@uu.nl
Walker,Jill,	GlaxoSmithKline,jill.t.walker@gsk.com
Walpita,Pramila	School of Medicine, University of Hawaii at Manoa,walpitap@hawaii.edu
Wang,Yao	University of California, Los Angeles,wangyao@ucla.edu
Warlich,Sandra	Friedrich-Loeffler-Institut,sandra.warlich@fli.bund.de
Weber,Friedemann	Philipps-University Marburg,friedemann.weber@staff.uni-marburg.de
Weis,Michael	Institute of Virology, Philipps-University Marburg, Marburg, Germany,michael.weis@staff.uni-marburg.de
White,Kris	Icahn School of Medicine at Mount Sinai,kris.white@mssm.edu
Wichgers Schreur,Paul	Central Veterinary Institute of Wageningen UR,paul.wichgersschreur@wur.nl
Widjojoatmodjo,Myra	Crucell Holland,mwidjojo@its.jnj.com
Wiltzer,Linda	Monash University, Australia,linda.wiltzer@monash.edu
Wimmer,Eckard	Stony Brook University,eckard.wimmer@stonybrook.edu
Wolff,Svenja	Institute of Virology, Philipps-University Marburg, svenja.wolff@staff.uni-marburg.de
Wolff,Thorsten	Robert Koch-Institut,WolffT@RKI.de
Wolkerstorfer,Andrea	Savira pharmaceuticals GmbH,andrea.wolkerstorfer@savira.at
Yamaoka,Satoko	The United Graduate School of Veterinary Sciences, Gifu University, p5103403@edu.gifu-u.ac.jp
Yamauchi,Yohei	ETH Zurich, Zurich, Switzerland,yohei.yamauchi@bc.biol.ethz.ch
Yasuda,Jiro	Institute of Tropical Medicine, Nagasaki University,j-yasuda@nagasaki-u.ac.jp
Yoneda,Misako	University of Tokyo,yone@ims.u-tokyo.ac.jp
York,Ashley	Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, United Kingdom,ashley.york@path.ox.ac.uk
Yoshida,Asuka	Department of Virology, Institute of Biomedical & Health Sciences, Hiroshima University,asurinco1129@gmail.com
Yoshida,Reiko	Research Center for Zoonosis Control, Hokkaido University,ryoshida@czc.hokudai.ac.jp
Yu,Qin	AstraZeneca,qin.yu@astrazeneca.com
Yun,Nadezhda	Department of Pathology, Galveston National Laboratory, University of Texas Medical Branch, Galveston, TX , neyun@utmb.edu
Yun,Nadezhda	Department of Pathology, Galveston National Laboratory, University of Texas Medical Branch, Galveston, TX ,neyun@utmb.edu
Zahn,Roland	Crucell,rzahn@its.jnj.com

Zhang,Jason	The AIDS Vaccine Design and Development Laboratory, International AIDS Vaccine Initiative,xzhang@iavi.org
Zhang,Xinsheng	The AIDS Vaccine Design and Development Laboratory, The International AIDS Vaccine Initiative,xzhang@iavi.org
Zhou,Hongbo	Section of Virology, Department of Medicine, Imperial College London, St. Mary's Campus, London, United Kingdom,hongbo.zhou@imperial.ac.uk
Zhou,Yan	University of Saskatchewan,yan.zhou@usask.ca

“NSV2013 Granada is supported in part
by AstraZeneca and Zymo Research”

GRANADA


A


B