EUROPEAN SEMINARS in VIROLOGY

6th European Seminar in Virology (EuSeV)

University of Bologna Residential Center in **Bertinoro, Italy**

June 22-24, 2018

Virus-host interaction at single cell and organism level

Abstract book and program

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Virus-host interaction at single cell and organism level

Organizers:

Gabriella Campadelli-Fiume,
University of Bologna

Dana Wolf,
Hebrew University Jerusalem

Thomas Mertens,
Ulm University Medical Centre

on behalf of the European Society
for Virology (ESV)
President Giorgio Palù

6. European Seminars in Virology (EuSeV) 2018

Program

FRIDAY 22.06.2018	
16:00-16:15	**Welcome** **Dana Wolf, Gabriella Campadelli-Fiume, Thomas Mertens, Giorgio Palù**
Virus-host-interactions at organism and cell level **Chair: Gabriella Campadelli-Fiume and Giorgio Palù**	
16:15-16:45	Pasetti, Marcela and Zachos, Nicholas Modeling Rotavirus Infection and Maternal Immunity in Human Enteroids Dept. of Pediatrics, Center of Vaccine Development, University of Maryland, School of Medicine, 655 W. Baltimore ST, Baltimore, MD 21201, USA, mpasetti@som.umaryland.edu John Hopkins University, Baltimore, MD 21201, USA
16:45-17:15	Wolthers, Katja The age of organoids: new ways for virus host interaction studies Dept. of Medical Microbiology AMC Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam-Zuidoost, The Netherlands, k.c.wolthers@amc.uva.nl
17:15-17:45	Ciuffi, Angela Single-cell analyses applied to HIV Institute of Microbiology, Lausanne University Hospital and University of Lausanne, Bugnon 48 – CHUV 1011 Lausanne, Switzerland, angela.Ciuffi@chuv.ch
17:45-18:15	Greber, Urs Mechanisms in cell-to-cell variability of virus infection Institute of Molecular Life Sciences, University of Zurich, Winterthurerstr. 190 8057 Zurich, Switzerland, urs.greber@imls.uzh
18:15-18:30	**Gat-Viks Irit** Dissection of influenza infection *in vivo* by single-cell RNA sequencing School of Molecular Cell Biology and Biotechnology, Dept. of Cell Research and Immunology, George S. Wise Faculty of Life Sciences, Tel Aviv University, 6997801 Tel Aviv, Israel, iritgv@post.tau.ac.il
18:30-18:45	**Manganaro Lara[1,5]**, Hong Patrick[1], Hernandez Matthew M.[1], Argyle Dionne[1], Mulder Lubbertus C.F.[1,3], Potla Uma[1], Diaz-Griffero Felipe[2], Lee Benhur[1,3], De Francesco Raffaele[5], Fernandez-Sesma Ana[1], Simon Viviana[1,3,4] IL-15 modulates HIV resistance through SAMHD1 inactivation in CD4+ T cells [1]Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, New York, USA, manganaro@ingm.org [2]Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY 10461, USA [3]Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New York, New York, USA [4]Division of Infectious Diseases, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, New York, USA [5]INGM, Istituto Nazionale Genetica Molecolare "Romeo ed Enrica Invernizzi", Milan, Italy
18:45-19:00	**Yaakov Liran Ben**, Mutsafi Yael, Porat Ziv, Dadaosh Tali, Minsky Avraham. Exploring host viral interactions during Mimivirus infection using quantitative analyses Department of Structural Biology, Weizmann Institute of Science, Rehovot, Israel, liran.ben-yaakov@weizmann.ac.il

DISCUSSIONS IN FRONT OF POSTERS

Lytic reactivation of KSHV is associated with major nucleolar alterations

Atari Nofar, K. Shanmugha Rajan, Vaibhav Chikne, Shulamit Michaeli and Ronit Sarid
The Mina and Everard Goodman Faculty of Life Sciences and Advanced Materials & Nanotechnology Institute, Bar Ilan University, Ramat-Gan, Israel, nahum.nofar@gmail.com

Herpesvirus infections in KIR2DL2 positive Multiple Sclerosis patients: mechanism triggering autoimmunity?

Bortolotti Daria[1], Sicolo Mariangela[1], Baldi Eleonora[2], Pugliatti Maura[3], Bortoluzzi Alessandra[4], Govoni Marcello[4], Fainardi Enrico[5], Di Luca Dario[1], Rizzo Roberta[1]:
[1]University of Ferrara, Department of Medical Sciences, Ferrara, Italy, brtdra@unife.it
[2]University Hospital, Arcispedale S. Anna, Department of Neurosciences and Rehabilitation, I-Ferrara
[3]University of Ferrara, Department of Biomedical and Specialty Surgical Sciences, I-Ferrara
[4]University Hospital, Arcispedale S. Anna, Department of Medical Sciences, I-Ferrara
[5]University Hospital, Careggi, Department of Diagnostic Imaging, I-Florence

In silico structural modeling of Hepatitis C virus fusion machinery: insights into immune evasion and entry

Castelli, Matteo
Microbiology & Virology laboratory, Universita' Vita-Salute San Raffaele, I-Milan, castelli.matteo@hsr.it

Muscle weakness associated with H7N9 infection: report of two cases

Chao-Nan Jin
State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, Zhejiang, Province,China, jincn@zju.edu.cn

Cell-specific adaptation of West Nile virus following serial passages in cell cultures from vertebrate host and invertebrate vector

Cosseddu Gian Mario, DiBiase Letizia, Marcacci Maurilia, Leone Alessandra, Pinoni Chiara, Patavino Claudio, Orsini Massimiliano, Monaco Frederica
Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Campo Boario, 64100 I-Teramo, g.cosseddu@izs.it

Ex vivo modeling of Human Cytomegalovirus nasal entry site

From Ido[1,2,3], Alfi Or*[1,2,3], Yakirevitch Arkadi[4], Wolf Michael[4], Weisblum Yiska[1,2,3], Zakay-Rones Zichria[2], Oberbaum Menachem[5], Panet Amos[2], Wolf Dana G.[1,3]
[1]Clinical Virology Unit, Hadassah University Hospital, Jerusalem, Israel, from.ido@mail.huji.ac.il
[2]Dept. of Biochemistry, IMRIC, The Hebrew University Faculty of Medicine, Jerusalem, Israel,
[3] Lautenberg Center for General and Tumor Immunology, Jerusalem, Israel, [4]Dept. of Otolaryngology Head and Neck Surgery, Sheba Medical Center, Tel Hashomer, Israel
[5]The Center for Integrative Complementary Medicine, Shaare Zedek Med. Center, Jerusalem, Israel

Towards the identification and characterization of host factors in Adenovirus entry

Gómez Alfonso, Bauer Michael, Hemmi Silvio, Greber Urs F.
Deptartment of Molecular Life Sciences, University of Zurich, Zurich, Switzerland, alfonso.gomezgonzalez@uzh.ch

Elucidating the relationship between molecular structure and antiviral activity of cationic amphiphilic drugs

Gunesch Antonia P.[1,2,3], Zapatero-Belinchon Francisco J.[1,2,3], Manns Michael P.[2,3], Steinmann Eike[3,4], Brönstrup Mark[3,5], Schneider Gisbert[6], von Hahn Thomas[1,2,3]
[1]Institute for Molecular Biology, Hannover Med. School, Hannover, Germany,
[2]Dept. of Gastroenterology, Hepatology and Endocrinology, Hannover Med. School, Hannover, Germany, gunesch.antonia-patrizia@mh-hannover.de
[3]German Center for Infection Research (DZIF), Hannover-Braunschweig site, Germany;
[4]Institute of Exp. Virology, TWINCORE, Center for Experimental & Clinical Infection Research, Hannover, Germany,
[5]Dept. of Chemical Biology, Helmholtz Centre for Infection Research (HZI), Braunschweig, Germany;
[6]Dept. of Chemistry and Applied Biosciences, Institute of Pharmaceutical Sciences, ETH Zürich, Switzerland

Avian leukosis virus receptors and biotechnological approach to host

resistance

Hejnar Jiří[1], Plachý Jiří[1], Trefil Pavel[2], Koslová Anna[1], Reinišová Markéta[1], Kučerová Dana[1], Mucksová Jitka[2], Kalina Jiří[2]
[1]Institute of Molecular Genetics, Czech Acad. of Sciences, Prague 4, Czech Rep., hejnar@img.cas.cz
[2]BIOPHARM, Research Institute of Biopharmacy and Veterinary Drugs, Jilove u Prahy, Czech Rep.

Population heterogeneity of Varicella-zoster virus with different passages in cell culture

Hwang Hye Rim, **Kang Ji Hye**, Kim Seok Cheon, Yeon Sang Hoon, Lee Il Seop, Jeon Jeong Seon, Lee Chan Hee
Department of Microbiology and Department of Computer Science, Chungbuk National University, Cheongju, South Korea, jihea7474@naver.com

Borna disease virus utilizes IGF2BP2 for translational regulation

Makino Akiko[1,2], Yamamoto Yutaro[2], Hirai Yuya[3], Tomonaga Keizo[1,2]
[1]Laboratory of RNA viruses, Institute for Frontier Life & Medical Sciences, Kyoto University
[2]Dept. of Mammalian Regulatory Network, Graduate School of Biostudies, Kyoto University
[3]Dept. of Biology, Osaka Dental University, Japan
makino@infront.kyoto-u.ac.jp

CD81 positive exosomes play a pivotal role in establishment of persistent infection in chronic HCV patients

Malik Maliha Ashraf, Ishtiyaq Javeria, **Manzoor Sobia**
Atta-ur-Rehman School of Applied Biosciences, National University of Sciences and Technology, Islamabad, Pakistan, dr.sobiamanzoor@asab.nust.edu.pk

Towards understanding the role of sphingolipids and the unfolded protein response in lytic virus egress from Adenovirus-infected cells

Martínez López Itzel Shantal, Prasad Vibhu, Suomalainen Maarit, Greber Urs F.
Institute of Molecular Life Sciences, University of Zurich, Switzerland, itzel.martinezlopez@imls.uzh.ch

Cellular localization of the proteins of the region 3 of feline enteric Coronavirus

Mészáros István[1], **Olasz Ferenc**[1], Kádár-Hürkecz Enikő[1,2], Támas Vivien[3], Bálint Ádám[3], Hornyák Ákos[3], Belák Sándor[4], Zádori Zoltán[1]
[1]Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary, olasz.ferenc@agrar.mta.hu
[2]National Institute of Environmental Health (NIEH), Budapest, Hungary
[3]National Food Chain Safety Office Veterinary Diagnostic Directorate, Budapest, Hungary
[4]Swedish University of Agricultural Sciences (SLU), Department of Biomedical Sciences and Veterinary Public Health (BVF), Uppsala, Sweden

The SAT protein of Porcine Parvovirus accelerates ER stress and changes the localisation of the pro-apoptotic CHOP in the infected cells

Mészáros István[1], Olasz Ferenc[1], Tijssen Peter[2], Zádori Zoltán[1]
[1]Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences. 21 Hungária krt., Budapest 1143, Hungary, meszaros.istvan@agrar.mta.edu
[2]INRS-Institut Armand-Frappier, Université du Québec. 531 Boulevard des Prairies, Laval, QC H7V 1B7, Canada

Snapweed as a reservoir of Cucumber mosaic virus in the region of Kaniv Natural Reserve

Novitska Victoria, Budzanivska Irena
Virology department, Education and Scientific Center Institute for Biology and Medicine, Taras Shevchenko National University of Kyiv, Ukraine, victorianovitska@gmail.com

20:00	**Dinner at outside Restaurant**

SATURDAY 23.06.2018

Virus-host-interactions at organism and cell level

Chair: Dana Wolf and Ben Berkhout

08:30-09:00	Berkhout, Ben Humanized mouse model of HIV. Laboratory of Exp. Virology, Dept. of Med. Microbiology, Academic Med. Center of the University of Amsterdam, Meibergdreef 14, K3-110 1105 AZ Amsterdam, The Netherlands, b.berkhout@amc.uva.nl
09:00-09:30	Metzner, Karin Unravelling HIV-1 latency, one cell at a time Department of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, Rämisstr. 100, 8091 Zurich, Switzerland, karin.metzner@usz.ch
09:30-10:00	Pagani, Massimiliano Single cell approaches to study the immune system Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Via Francesco Sforza 35, 20122 Milano, Italy, massimiliano.pagani@unimi.it
10:00-10:30	Rosenberg, Brad Single cell transcriptomics for characterizing the human immune response to yellow fever virus Icahn School of Medicine at Mount Sinai Annenberg Bldg. 17-70C, 1468 Madison Ave, New York, NY 10029, brad.rosenberg@mssm.edu
10:30-11:00	**Break**
Virus evolution and dynamics **Chair: Michael Kann and Esteban Domingo**	
11:00-11:30	Domingo, Esteban Confronting RNA viruses: from quasispecies to lethal mutagenesis. Centro de Biología Molecular Severo Ochoa (CSIC-UAM), c/Nicolás Cabrera, 1 Campus de Cantoblanco Universidad Autónoma de Madrid, 28049 Madrid, Spain, edomingo@cbm.csic.es
11:30-12:00	Vignuzzi, Marco Monitoring, predicting and targeting virus evolution and transmission Viral populations and Pathogenesis Unit, CNRS UMR 3569, Institut Pasteur, 28, rue du Dr. Roux, 75724 Paris cedex 15, marco.vignuzzi@pasteur.fr
12:00-12:15	Mutuel Doriane[1], Pigeyre Laetitia[1], Nam Ki-Woong[1], Gosselin Grenet Anne-Sophie[1], Froissart Rémy[2], **Ogliastro Mylène[1]** Densovirus experimental evolution *in insecta*: the appearance can be deceptive [1]INRA, UMR 1333, Diversité, Génomes & Interactions Microorganismes-Insectes, Montpellier, France, marie-helene.ogliastro@inra.fr [2]CNRS, UMR 5290 MIVEGEC, F-34394, Montpellier, France.
12:15-14:00	**Lunch break** **(Meeting of the EB and AC of ESV)** **DISCUSSIONS IN FRONT OF THE POSTERS**
Regulation I **Chair: Veronika von Messling and Lynn Enquist**	
14:00-14:30	Enquist, Lynn W. Silenced or productive infection? Engagement of an alphaherpesvirus with peripheral nervous system neurons Department of Molecular Biology, Princeton University, 119 Lewis Thomas Laboratory Washington Road, Princeton, NJ 08544-1014, USA, lenquist@princeton.edu
14:30-15:00	Dutch, Rebecca Entry, replication and spread of negative strand viruses: Lessons from Human Metapneumovirus Dept. of Molecular & Cellular Biochemistry, University of Kentucky, Coll. of Med., BBSRB Bldg., 741 S Limestone, B148b, Lexington, KY 40536-0509, rdutc2@uky.edu
15:00-15:15	Blondot Marie-Luise, **Gallucci Lara**, Kann Michael Single molecule DNA labeling system to follow the uncoating step of the Hepatitis B virus

	CNRS UMR 5234, Microbiologie Fondamentale et Pathogénicité, Université de Bordeaux, Bordeaux, France lara.gallucci@u-bordeaux.fr
15:15-15:30	Fernández de Castro Martin Isabel[1], Fournier Guillaume[2], Sachse Martin[3], Pizarro-Cerda Javier[4], Risco Cristina[1], **Naffakh Nadia[2]** Influenza virus genome reaches the plasma membrane via a modified endoplasmic reticulum and Rab11-dependent vesicles [1]Centro Nacional de Biotecnologia, CNB-CSIC, Cell Structure Lab, Madrid, Spain [2]Institut Pasteur, Unité Génétique Moléculaire des Virus à ARN, CNRS UMR3569, Université Paris Diderot, Paris, France, nadia.naffakh@pasteur.fr [3]Institut Pasteur, Ultrapole, Paris, France [4]Institut Pasteur, Unité des Interactions Bactéries-Cellules, Paris, France
15:30-15:45	**Nambala Peter[1]**, Su Wen-Chi[1,2] The identification of post translational modifications on the Zika virus precursor membrane protein and their roles in virus infectivity [1]Graduate Institute of Biomedical Sciences (MSc Program), China Medical University, Taichung, Taiwan, peternambala@hotmail.com [2]Research Center for Emerging Viruses, China Medical University Hospital, Taichung, Taiwan
15:45-16:00	**Bigi Rachele**, An Hyowon, Raab-Traub Nancy, Dittmer Dirk P. Epstein-Barr virus (EBV) enhances plasmid maintenance of Kaposi Sarcoma-associated Herpesvirus (KSHV) Lineberger Comprehensive Cancer Center, The University of North Carolina, 450 West Drive, Chapel Hill, NC 27599, US, bigi@email.unc.edu
16:00-16:30 BREAK	
Regulation II **Chair: Rebecca Dutch and Angela Ciuffi,**	
16:30-16:45	**Declercq Marion**, Biquand Elise, Karim Marwah, Demeret Caroline, Barbezange Cyril, van der Werf Sylvie Screening for cellular exoribonucleases interacting with the Influenza A virus polymerase suggests a role of cellular protein ERI1 in the virus life cycle Unité Génétique Moléculaire des Virus à ARN, UMR3569 CNRS, Université Paris Diderot Sorbonne Paris Cité, Département de Virologie, Institut Pasteur, Paris, France, marion.declercq@pasteur.fr
16:45-17:00	Derewońko Natalia, Panasiuk Miroslawa, Rychłowski Michal, **Bienkowska-Szewczyk Krystyna** Direct cell-to-cell spread is the preferred mode of alpha-herpesviruses transmission in non-transformed cells University of Gdansk, Intercollegiate Faculty of Biotechnology, Dept. Molecular Virology, Gdansk, Poland, krystyna.bienkowska-szewczyk@biotech.ug.edu.pl
17:00-17:15	**Gonzalez-Reiche Ana S**.[1], Peralta Zuleyma[1], Fenouil Romain[1], Rialdi Alexander[2], Zhao Nan[2], Lagda Arvin[2], O'Hanlon Ryan[2], Kimaada Alette[1], Sebra Robert[1], Shaw Megan L.[1], Marazzi Ivan[2], van Bakel Harm[1] Analysis of Influenza A virus transcription at the single cell level [1]Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York City, NY, USA, anasilvia.gonzalez-reiche@mssm.edu [2]Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA
Immunity/Immune response I **Chair: Monsef Berkirane and Urs Greber**	
17:15-17:45	Benkirane, Monsef CD4 T cell 3D genome structure: Impact on HIV integration site selection and transcription Institut de Génétique Humaine/Virologie Moléculaire, CNRS-Université de Montpellier, 141 rue de la Cardonille, 34000 Montpellier, France, monsef.benkirane@igh.cnrs.fr
17:45-18:00	**D'Urbano Vanessa[1]**, Longo Serena[1], De Crignis Elisa[1], Bertoldi Alessia[1], Musumeci Giuseppina[1], Bon Isabella[1], Calza Leonardo[2], Gallinella Giorgio[3], Re Maria Carla[1]

	HIV-1 and host restriction factors: relationship between viral reservoir and RFs expression levels [1]Microbiology Section of the Department of Experimental, Diagnostic and Specialty Medicine, School of Medicine, University of Bologna, Italy, vanessa.durbano3@unibo.it [2]Clinic of Infectious Diseases, Department of Medical and Surgical Sciences, St. Orsola-Malpighi Hospital, University of Bologna, Italy [3]Department of Pharmacy and Biotechnology, University of Bologna, Italy
18:00-18:15	Gulimov Mikhail K., **Ammour Yulia I.**, Astapenko Anastasia V., Romantsova Lyudmila R., Svitich Oksana A., Zverev Vitaly V. Molecular crosstalk between autophagy and Rubella virus Mechnikov Research Institute for Vaccines and Sera, Moscow, Russia, 105064, yulia.ammour@yahoo.fr
18:15-18:30	**Kazungu Yvette**, Carletti Tea, Zakaria Khalid Mohamed and Marcello Alessandro The intimate relationship between the UPR and innate immunity following Flavivirus infection. Laboratory of Molecular Virology, International Centre for Genetic Engineering and Biotechnology, Trieste, Italy, alizeyvette@yahoo.com
19:15	Bus transfer to Dinner at outside Restaurant

SUNDAY 24.06.2018	
Immunity/Immune response II **Chair: Marlène Dreux and Richard E. Randall**	
08:30-09:00	Dreux, Marlène Flavor of Flavivirus by plasmacytoid dendritic cells CIRI, Inserm U1111 CNRS UMR 5308, 21 Avenue Tony Garnier, 69365 Lyon, France, marlene.dreux@ens-lyon.fr
09:00-09:30	Randall, Richard E. Paramyxoviruses, interferon and persistence; variations at the molecular, viral, cellular and organism levels influence the outcomes of infection University of St. Andrews, School of Biology, BMS Bldg., North Haugh, St. Andrews, Fife, KY16 9ST, UK, rer@st-andrews.ac.uk
09:30-09:45	**Mantel Nathalie**[1], Piras-Douce Fabienne[1], Raynal Franck[1], Marcos-Lopez Ernesto[2], Chautard Emilie[1], Courtois Virginie[1], Dereuddre-Bosquet Nathalie[2], Le Grand Roger[2] Safety and immuno-efficacy profiles of the Yellow Fever vaccine versus wild-type infection in the Cynomolgus macaques (Macaca fascicularis), [1]Sanofi Pasteur, Marcy l'Etoile, France, nathalie.mantel@sanofi.com [2]CEA, INSERM U1184, Université Paris-Sud 11, IDMIT Department, Fontenay aux Roses, France
09:45-10:00	**Nicoli Francesco**[1,2,3], Gallerani Eleonora[2], Sforza Fabio[2], Finessi Valentina[2], Chachage Mkunde[4], Geldmacher Christof[5], Cafaro Aurelio[6], Ensoli Barbara, Gavioli Riccardo[2], Caputo Antonella[1] The HIV-1 Tat protein affects human CD4+ T-cell programming and activation, and favors the differentiation of naïve CD4+ T cells. [1]Department of Molecular Medicine, University of Padova, Padova, Italy, nclfnc1@unife.it [2]Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy [3]Center for International Health, Ludwig Maximilians University, Munich, Germany [4]National Institute for Medical Research (NIMR)-Mbeya Medical Research Centre, Mbeya, Tanzania [5]Division of Infectious Diseases and Tropical Medicine, Medical Center of the University of Munich (LMU), Munich, Germany, [6]AIDS Center, Istituto Superiore di Sanità, Rome, Italy
10:00-10:15	**Rand Ulfert**, Kubsch Tobias, Kasmapour Bahram, Cicin-Sain Luka Virus-host interactions at the single-cell level – CMV gene expression

	dynamics and the innate immune response Helmholtz Centre for Infection Research, Braunschweig, Germany, ulfert.rand@helmholtz-hzi.de
Disease and Therapy **Chair: Monique Lafon and Thomas Mertens**	
10:15-10:45	Lafon, Monique Takeover by rabies virus G protein of signaling pathways driving neuron survival: a source of innovative therapeutical molecules for neurodegenerative diseases Department of Virology, Institut Pasteur-Paris, 25, rue du Dr. Roux, 75724 Paris, France monique.lafon@pasteur.fr
10:45-11:00	Gonzalez Gaelle, Aulner Nathalie, Fares Mazigh, Cochet Marielle, Montéro-Ménei Claudia, Anne Danckaert Anne, **Coulpier Muriel** High content screening/imaging of differentiated human primary neural cells identifies drug candidates that inhibits TBEV infection UMR 1161 INRA-ANSES-ENVA Virology, Neurovirology of Zoonoses, Ecole Nationale vétérinaire d'Alfort, Maisons-Alfort, France, muriel.coulpier@vet-alfort.fr
11:00-11:15	**Singh Harvijay**, Narwal Manju, Mudgal Rajat, Kumar Pravindra, Tomar Shailly Inhibition of Chikungunya virus nsP2pro cysteine protease by structure-based peptidomimetics antivirals. Dept. of Biotechnology, Indian Institute of Technology Roorkee-247667, India, harvijay.iitr@gmail.com

BRUNCH	
12.00	**Bus leaves for Bologna Airport**

Abstracts and posters in alphabetical order of authors

Lytic reactivation of KSHV is associated with major nucleolar alterations

Nofar Atari, K. Rajan Shanmugha, Vaibhav Chikne, Shulamit Michaeli and Ronit Sarid
The Mina and Everard Goodman Faculty of Life Sciences and Advanced Materials & Nanotechnology Institute, Bar Ilan University, Ramat-Gan, Israel

The nucleolus is a subnuclear compartment whose primary function is biogenesis of ribosomal subunits. Previous studies have shown that certain viral infections affect the morphology and composition of the nucleolar compartment and influence rRNA transcription and maturation. However, no description of the nucleolar morphology and function during infection with Kaposi's sarcoma-associated herpesvirus is available to date.

By using immunofluorescence microscopy, we have documented an extensive destruction of the nuclear and nucleolar architecture during lytic reactivation of KSHV. Redistribution of key nucleolar proteins, including the rRNA transcription factor UBF, the essential pre-rRNA processing factor Fibrillarin, and the nucleolar phosphoprotein Nucleophosmin, was documented. Of note, distinct delocalization patterns were evident. Fluorescent *in situ* hybridization, combined with immunofluorescence, revealed a complete overlap between Fibrillarin and ITS-1, which represents the primary product of rDNA, whereas the dispersion pattern of UBF and ITS-1 only partially overlapped. A complete co-localization of UBF and the RNA polymerase I subunit RPA194 was observed, while UBF did not co-localize with Fibrillarin. No accumulation of pre-rRNA intermediates was evident by Northern blot analysis, suggesting that processing of pre-rRNA proceeds properly.

Taken together, our results suggest that rRNA transcription and processing persist during lytic reactivation of KSHV, yet they appear to be uncoupled. Whether the observed nucleolar alterations favor productive infection or signify cellular anti-viral responses remain to be determined.

CD4 T cell 3D genome structure: Impact on HIV integration site selection and transcription

Monsef Benkirane
Institut de Génétique Humaine/Virologie Moléculaire, CNRS-Université de Montpellier, Montpellier, France

Humanized mouse model of HIV

Ben Berkhout
Laboratory of Experimental Virology, Department of Medical Microbiology, Academic Medical Center of the University of Amsterdam, Amsterdam, The Netherlands

The study of candidate HIV drugs and novel therapeutic approaches like gene therapy requires the availability of an animal model. Whereas one could consider the SIV-macaque model, it is expensive, raises ethical concerns and SIV is not the same as HIV. For these reasons, humanized mouse models have been set up. We use immuno-incompetent mice that receive human fetal blood stem cells that develop in the course of a month in mature immune cells, e.g. T and B cells. This provides a valuable tool for HIV replication studies and testing of antiviral drugs. We will also demonstrate the usefulness of this in vivo model for the evaluation of an anti-HIV gene therapy approach that is very promising in simple in vitro systems.

Epstein-Barr virus (EBV) enhances plasmid maintenance of Kaposi Sarcoma-associated Herpesvirus (KSHV)

Rachele Bigi, Hyowon An, Nancy Raab-Traub and Dirk P. Dittmer
Lineberger Comprehensive Cancer Center, The University of North Carolina, 450 West Drive, Chapel Hill, NC 27599 (bigi@email.unc.edu)

Primary effusion lymphoma (PEL) is a B-cell lymphoma, which is always associated with Kaposi's Sarcoma-associated herpesvirus (KSHV) and in many cases also with Epstein-Barr virus (EBV); however, the requirement for EBV co-infection is not clear. We find that adding EBV genomes to $KSHV^+$ single positive PEL lead to increased KSHV genome maintenance and KSHV Latency-Associated Nuclear Antigen (LANA) expression. In naturally co-infected PEL, EBV was necessary for KSHV plasmid maintenance. When $KSHV^+/EBV^+$ PEL were transfected with EBV- CRISPR/Cas9 plasmids targeting different regions in EBV to deplete the viral genome, we observed a dramatic decrease in cell viability, KSHV genome copy number and LANA protein expression. Ectopic expression of EBNA-1 ameliorated this effect, even though LANA and EBNA-1 did not colocalize. This implies that EBV plays an essential role in the pathogenesis of PEL by increasing KSHV viral load and LANA expression. It also suggests that small molecules that target EBV EBNA-1 may have efficacy against dually infected PEL.

Single molecule DNA labeling system to follow the uncoating step of the Hepatitis B virus

Marie-Lise Blondot, **Lara Gallucci** and Michael Kann
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Despite of a vaccine, chronic Hepatitis B Virus (HBV) infection remains a major global threat, causing 600,000 deaths each year. The development of therapies is challenged by the lack of suitable animal and cellular models, due to the narrow host range and the restricted tropism of the virus. Species specificity is determined by host factors at different levels of the viral life cycle, which starts with the binding to the HBV receptors Na+taurocholate cotransporting polypeptide (NTCP) and heparan sulfate polyglycanes. The viral capsid, comprising the relaxed circular viral DNA (rcDNA), is then transported through the cytoplasm and nuclear pore into the nuclear basket. There, the capsid is arrested due to interaction with Nup153 and disassembles, leading to nuclear rcDNA release and subsequent repair to an episomal form, called circular covalently closed DNA (cccDNA). Noteworthy, this release is restricted to capsids with a mature genome. It has been reported that the expression of hNTCP in mouse hepatocytes is not sufficient for the formation of cccDNA although the hepatitis D virus, which uses the surface proteins of HBV, infects these cells successfully. This suggests the presence of a post entry, HBV- and species-specific restriction factor.

We identify the light chain 1 of the Dynein (DynLL1) as the binding partner of the capsid during the movement along the microtubules. Being the DynLL1 well conserved among species, the species-specific factor is narrowed to an event after capsid arrival in the nuclear basket, thus it is likely to be a protein involved in genome release and/or repair, since these two steps might be connected to each other.

We have developed a system allowing the study of the uncoating step by following the viral genome released in the nucleus of an infected cell.

The system is based on the cooperative binding of a fluorescent fusion protein to an anchor sequence. It consists of an infection model made by human hepatic cell lines expressing a bacterial green fluorescent protein and pseudo viruses harboring in their genome a target sequence for this protein. Upon infection, when the viral genome is released and becomes accessible, the bacterial protein binds to it leading to the appearance of green fluorescent spots in the nucleus. This DNA labeling system allows the detection of single double strand DNA molecules in living cells.

Using this read out, we were able to observe the release of the viral genome from the capsid and obtained kinetic data on the repair of the rcDNA. The use of chemical inhibitors targeting enzymes likely involved in uncoating and genome repair will now enable us to elucidate the underlying molecular basis and to identify the nature of species-specific factor different in mouse and humans.

Herpesvirus infections in KIR2DL2 positive Multiple Sclerosis patients: mechanism triggering autoimmunity?

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The major difficulty in associating Multiple sclerosis (MS) to Human Herpesviruses (HHVs) infection is proving that the infection comes prior to the autoimmune disease. We have reported that Killer Ig-like receptor (KIR)2DL2 inhibitory receptor expression on the surface of $CD56^{bright}$ Natural killer (NK) cells impairs the response to HHVs infections in a subgroup of MS patients[1-3]. NK cells may affect anti-viral response directly, causing infected cell lysis, or indirectly, stimulating B-cell responses in part through CD40/CD40L interaction or affecting T cells activation[4]. In pathological conditions, NK/B cell interactions could be modified by viral antigens, that present homologies with self-antigens. It is known that, HHV (Human herpesvirus)-6 pU24(1-15) peptide has a potential role in mimicking myelin basic protein (MBP)[5] in MS, with a potential effect on both cellular and humoral immune response. We aimed to analyze anti-HHVs specific antibody synthesis and the expression of CD40/CD40L molecules in MS patients, focusing on HHV-6 U24(1-15) petptide.

We enrolled 40 MS patients with a stable RRMS, 40 healthy controls and 40 patients with neuropsychiatric systemic lupus erythematosus (NLES). We analyzed HHVs (EBV, VZV, HHV-6, HSV-1, HSV-2) sierology (IgM/IgG) in peripheral serum and immune-phenotyped NK cells for CD56, KIR2DL2, CD69, CD154 expression and B cells for CD19, CD20, CD40 and CD27 expression. We investigated the effect of HHV-6 pU24(1-15) peptide on both humoral and cellular immunity evaluating sierology (IgG) and INF-gamma and Granzyme-B production by Fluorospot assay.

We found a higher frequency of the number of simultaneously-increased specific anti-herpesvirus IgG titers in the blood of MS and NLES patients in comparison with controls (N: 5 vs 2; $p<0.01$). Moreover, MS patients positive for KIR2DL2 expression on $CD56^{bright}$ NK cells demonstrated significant increased IgG titers towards HHV-6 compared to controls and NLES ($p<0.01$), that correlates also with higher EDSS scores. In particular, MS patients presented higher levels of IgG towards HHV-6 U24(1-15) peptide than controls ($p<0.01$), comparable to those observed towards the corresponding MBP(93-105) self-peptide (p=NS). Considering antibody production towards the phosphorylated form of U24(1-15) peptide (pU24), we reported higher IgG levels, in particular in KIR2DL2+ MS patients, in both plasma and CFS ($p<0.01$; $p<0.001$, respectively). No modifications in IgM titers were observed. When we stimulated NK and T cells from MS patients with pU24(1-15) peptide, we observed a higher activation status in KIR2DL2+ MS patients in terms of IFN-gamma production by T cells, but not of Granzyme-B by NK cells, compared to controls.

We observed no differences in CD40 expression on B lymphocytes among the three cohorts. A subset comparison of CD56brightKIR2DL2+ patients and controls demonstrated a significant decrease in CD40 expression on CD57+IgGD- (switched memory) B lymphocytes of MS patients ($p < 0.0001$). Conversely, $CD56^{bright}KIR2DL2^{+}$ NK cells from MS patients showed an increased expression of CD40L (CD154) ($p=0.02$), while no differences were observed in controls. In conclusion, these results suggest that the expression of KIR2DL2 receptor on $CD56^{bright}$ NK cells might affect not only NK cells functions[1-3] but also B cells production of anti-herpesvirus antibodies in MS patients, mainly anti-HHV-6 U24 (1-15) antibodies, possibly via CD40L up-modulation. Since pU24 (1-15) peptide has an amino acid motif with an elevated half time of dissociation (HTD: 24) from HLA-C1 molecules, significantly higher in comparison with MBP (93-105) peptide (HTD: 0.289), we hypothesized a possible effect of pU24 (1-15) peptide on NK cells via KIR2DL2/U24/HLA-C1 interaction and B cell autoreactivity via U24/MBP mimicry. As a proof of concept, pU24(1-15) is not able to efficiently activate NK cells, supporting the KIR2DL2/U24/HLA-C1 inhibitory function, but it activates a massive T cell IFN-gamma secretion, suggestive of a memory T cells expansion.

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In silico structural modeling of Hepatitis C virus fusion machinery: insights into immune evasion and entry

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Direct structural studies of hepatitis C virus (HCV) surface glycoproteins E1 and E2, the constituents of its entry machinery, are limited to truncated and modified constructs due to their flexibility and high content in glycosylation. Conversely, E1 and E2 full-length structures, either alone or in complex, are not characterized, hampering the identification of the domains involved in virus entry and immune escape and, consequently, the development of strategies to interfere with HCV entry.

The described model comprises the entire E1 and E2 ectodomains and was generated *in silico* exploiting both experimental data and computational predictions. In details, a full-length alanine scanning library covering 553 out of the 555 E1 and E2 residues was generated and tested in cytofluorimetry for the binding to a panel of conformational human monoclonal antibodies (mAb), leading to the identification of their epitopes as well as of the disulfide connectivity underlying E1E2 native conformation. The disulfide bridges, together with inter-atomic distance restraints derived from the *in silico* prediction of secondary, tertiary and quaternary structures, were used to generate *ab initio* a fully-glycosylated, covalently-bound, E1E2 structural model.

Our results define a direct relationship between HCV entry machinery structure and function, shedding light on the conformational changes required for fusion and the strategies the virus adopts to evade the immune system. Indeed, the model highlights how E2 hypervariable regions and glycosylations shield the entire fusion machinery from the host humoral response, provides a structural explanation for the elusive role of E2 HVR1 in protecting multiple, unrelated, neutralizing epitopes, and indicates how the E1E2 complex reacts to the timely binding of the cellular receptors in a multi-step mechanism leading to fusion.

In the absence of experimental high-resolution structures for the E1E2 heterodimer, the model improves the comprehension of HCV entry process, thus representing a step towards the rational design of immunogens and drugs inhibiting its early stages of infection.

Muscle weakness associated with H7N9 infection: report of two cases

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Background: Patients who are infected with avian influenza A (H7N9) virus frequently present with pneumonia and acute respiratory disorder syndrome, with high rates of intensive care unit admission and death, while neurological complications such as Guillain–Barré syndrome and intensive care unit-acquired weakness including critical illness polyneuropathy and myopathy are rarely reported before.

Case presentation: The study included two severe H7N9 pneumonia patients with non-immune diseases prior to the onset of virus infection. A 56-year-old female patient (case 1) and a 78-year-old female patient (case 2) were admitted because of fever, cough, chest tightness and shortness of breath, confirmed H7N9 infection soon after admission, followed by the development of acute respiratory distress syndrome and various fatal bacterial and fungal infection. Case 1 patients was found muscle weakness in all extremities when tried to withdraw the mechanical ventilator and case 2 the extracorporeal membrane oxygenation, which both of this condition prolonged the time of ventilator weaning. What's more, case 1 carried H7N9 virus for a prolonged time as 28 days, and both of them stayed in hospital for a prolonged phase as more than two months. A clinical diagnosis of ICU-acquired weakness can be confirmed while according to results of electrophysiological testing and needle electromyography it's hard to differentiate critical illness polyneuropathy or Guillain–Barré syndrome, since no lumbar puncture, muscle and nerve biopsy were conducted in hospitality.

Conclusions: Though there's great improvement of possibility in saving severe patients' lives from fatal respiratory and blood infections, it's necessary to pay enough attention and take more methods to differentiate this complication as muscle weakness reported here, which results in ventilator-associated pneumonia and a prolonged hospital stay, further increases death rate, huge costs of medical and economic resources. Here, we report on two Chinese patients presenting H7N9 infection complicated by muscle weakness.

Single cell analysis applied to HIV

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Single-cell analyses provide a powerful tool to study virus-host interactions, providing key insights to specific cellular features involved in permissiveness or resistance, but also to persistence. Indeed, despite effective treatment, HIV can persist in latent reservoirs, which represent a major obstacle towards HIV eradication. Targeting and reactivating latent cells is challenging due to the heterogeneous nature of HIV infected cells. We used a primary model of HIV latency and single-cell RNA sequencing to characterize transcriptional heterogeneity during HIV latency and reactivation, and to understand transcriptional programs leading to successful reactivation of HIV expression.

Cell-specific adaptation of West Nile virus following serial passages in cell cultures from vertebrate host and invertebrate vector

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West Nile virus (WNV) is a mosquito-borne flavivirus identified for the first time in Italy in 1998. WNV is maintained in the environment alternating viral replication cycles in mosquitoes (vector) and birds (reservoir host). Mammals could be infected in a spillover transmission, but are considered "dead-end" hosts. WNV infection can cause serious neurological illness in humans and horses. Mechanisms of persistence and ability to become endemic rely on high plasticity of virus genome. However despite the remarkable mutational power, the ability to adapt to diverse host species is limited since WNV evolution strategy is maintaining adequate replicative fitness in two disparate hosts instead of gain a superior fitness in one single host (the trade-off hypothesis). Understanding the selective pressures that drive WNV adaptation and evolution in its disparate mosquito and avian hosts is crucial to predicting the ability to persist and re-emerge.

In this study two strains of WNV isolated from field in Italy, one belonging to lineage 1 (WNV_L1) and the other to lineage 2 (WNV_L2), were serially passaged for 10 times in mosquito (C6/36) and avian (DEF) cell lines. Viral RNA was extracted from cell culture supernatant and whole genome sequencing was performed on the NextSeq 500 (Illumina). After quality control, reads were trimmed and mapped to the reference genomes. Genetic changes resulting from passages of WNV_L1 and WNV_L2 in C6/36 and DEF cells were identified in nucleotide sequences of the full-length genomes comparing un-passaged and passaged viruses. The overall number of nucleotide changes observed after passages of WNV_L1 and WNV_L2 in C6/35 and DEF were similar. However cell specific mutation patterns were generated in mosquito and in avian cells, in fact majority of the nucleotide changes observed in WNV genome following passages in DEF cells were different from those observed in C6/36 and vice versa. Some mutations were found in both cell lines. Results clearly indicate that mosquito and avian cells do not constitute similar environments for WNV replication and are able to constrain WNV evolution.

Screening for cellular exoribonucleases interacting with the Influenza A virus polymerase suggests a role of cellular protein ERI1 in the virus life cycle

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The influenza A virus genome is composed of eight negative-stranded RNA segments, each encapsidated by nucleoproteins (NP) and associated to a trimeric polymerase complex (PB1, PB2 and PA) to form viral ribonucleoproteins. Their segmented genome nature and low polymerase fidelity provide influenza viruses with great genetic flexibility and easy adaptation to new hosts. The viral polymerase is critical for host restriction and is known to interact with an extensive network of cellular proteins. Mutations in the viral polymerase have been reported to modulate replication fidelity. However, no study has investigated the role of cellular proteins in influenza viruses replication fidelity. We thus selected a set of 98 proteins carrying exo(ribo)nucleases activities and, using a split luciferase assay, looked for their interactions with 7 viral proteins involved in viral replication for two influenza virus strains: A/Paris/650/2004(seasonal H1N1) and A/Bretagne/7608/2009(H1N1pdm09). Based on their interaction with at least one viral protein, 21 proteins were selected. Interaction profiles of the two strains showed little differences which indicated that identified interactions were well-conserved among influenza viruses. Upon siRNA knock down, viral titers with seasonal H1N1 or H1N1pdm09 were significantly decreased for 12 of the 21 proteins. Furthermore, knock down of 7 of these 12 proteins was associated with a decrease in the amount of viral proteins accumulated upon single cycle infection with H1N1 WSN (A/WSN/33(H1N1)). Among those 7 proteins, the exoribonuclease ERI1, shows a particularly strong decrease in viral particles production as well as in viral protein accumulation upon siRNA knock down. Remarkably, ERI1 interacts with PB2, PB1 and NP suggesting that it might be interacting with the trimeric viral polymerase. This interaction profile was further confirmed by pull down of ERI1 in H1N1 WSN infected cells underlying functional significance of this interaction during the viral cycle. Functional analysis of the role of ERI1 for influenza A virus replication is currently ongoing.

Direct cell-to-cell spread is the preferred mode of alpha-herpesviruses transmission in non-transformed cells

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For many animal enveloped viruses (HIV, measles virus) cell-to-cell spread provides the fast way of transmission from infected to non-infected cell and the opportunity to escape from the immune defense of the host. For herpesviruses, which after primary infection, are staying in infected organism for the entire life of their hosts, this strategy may be essential for successful reactivation and spread. It has been shown that herpesviruses can move between cells via tight junctions, gap junctions and all kinds of intercellular connections including filopodia and recently discovered tunneling nanotubes. In the present report we analyzed the efficiency of cell-to-cell transmission of our model alpha-herpesvirus, bovine herpesvirus 1 (BoHV-1), between various types of cells. To distinguish between direct viral transmission mode and “free entry”, that is, the access of free virus from the outside of the cell, we compared the course of infection under semisolid overlay (which limits the spread of viral particles but does not block virus entry from outside) and under liquid medium containing neutralizing antibodies which completely inhibited free entry. We performed standard plaque assay and compared the results with a new quantitative method of cell-to-cell analysis, allowing to calculate the number of newly infected cells during the time of plaque formation. These two methods showed that cell-to-cell was the favored mode of spread in primary cells in contrast to immortalized cell lines where free entry was necessary for plaque formation. Moreover, taking advantage of the availability of several fluorescent BoHV-1 mutants constructed in our lab, we investigated the interaction between individual live infected and non-infected cells. We observed that BoHV-1 infection stimulates cells to produce long intercellular projections (tunneling nanotubes, TNTs) utilized by the virus for the transfer between cells. The frequency of TNTs formation was higher in non-transformed cells and we observed virus transfer via intercellular bridges between different types of cells, e.g. epithelial cells and fibroblasts. Our observations of mutant virus infections confirmed the essential role of glycoprotein gE in cell-to-cell spread but also demonstrated that viral kinase Us3 is required for efficient intracellular trafficking of gE. Lack of BoHV-1 Us3 or impairing its kinase activity resulted in the decrease of BoHV-1 induced TNTs number and length. All our observations indicate that efficient cell-to-cell spread contributes significantly to natural course of herpesvirus infection. Thus, the mechanisms involved in this transmission route can be targeted for the development of antiviral therapies.

Confronting RNA viruses: from quasispecies to lethal mutagenesis

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The complex mutant spectra of RNA viruses represent an important challenge for the control of pathogenic viruses, but also an opportunity of new antiviral strategies. Mutant spectra are rapidly generated as a consequence of elevated mutation rates, and minority genomes are a phenotypic reservoir for adaptation. Several improvements for the control of viral disease have been derived from the understanding of quasispecies dynamics, notably the recognition of the need of multi-epitopic vaccines, and combination therapies. A new antiviral strategy is lethal mutagenesis, or extinction of viruses by an excess of mutations. It follows from one of the equations relevant to quasispecies theory: the error threshold relationship that has been also formulated as an extinction threshold for RNA viruses. Several base and nucleoside analogues are currently investigated as antiviral agents against pathogenic RNA viruses such as influenza virus, norovirus, West Nile, Zika or Ebola viruses, among others. The molecular basis of lethal mutagenesis will be illustrated with the evolution of a clonal population hepatitis C virus in a constant biological environment, and extinction by the mutagenic activity of the purine analogues favipiravir and ribavirin. Use of multiple diversity indices has helped understanding the nature of mutant spectrum modifications associated with extinction or resistance to extinction. Limitations and prospects of application of lethal mutagenesis to antiviral pharmacology will be discussed. The journey between quasispecies and lethal mutagenesis will be briefly evaluated to illustrate that basic research may lead to practical applications often in an unpredictable manner.

Flavor of Flavivirus by plasmacytoid dendritic cells

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Type I interferon (IFN-I) signaling is pivotal for the host control of viral infections, nonetheless many viruses evolved mechanisms to evade this response in the cells that they infect. We and others uncovered an alternative sensing pathway mediated by the plasmacytoid dendritic cells (pDCs), which involves physical cell-cell contact with infected cells, a feature observed for evolutionary distant viruses. Here, using dengue and Hepatitis C virus, as viral models, we unraveled characteristics of this newly discovered aspect of the innate immunity. We showed that the contacts between pDCs and infected cells are remodeled into specialized territories. This cell polarity includes local accumulations of cell adhesion molecules, modified lipids and proteins known to modulate membrane dynamics and trafficking, along with cortical components. The endocytosis machinery also polarizes at contact, in agreement with our previous report revealing the cell-to-cell transmission of immunostimulatory RNA to pDCs via exosome-like vesicles. We propose that pDC/infected cell contact site represents a novel form of synapses specialized for vesicular-mediated transmission of viral immunostimulatory RNA, that we named “interferogenic synapse”. Live-imaging analyses further suggested that pDCs establish sustained contacts with infected cells as opposed to the shorter contacts found with uninfected cells. Consistently, the TLR7-induced signaling in pDCs promotes cell polarity and establishment of sustained contacts, highlighting positive feedback regulations. Since cell contact-dependent pDC activation is observed for evolutionary distant viruses, we propose that cell polarization at the contact is a primary functional ability of pDCs to efficiently respond to infected cells. Thus, the scanning of viral infection by establishment of cell-cell contacts could represent an original hallmark of immune surveillance leading to a potent IFN response localized at the site of infection, hereby limiting a systemic response deleterious to the host. Yet, the unique role of pDCs in regulating *in vivo* infection, especially in cases where they are not targets of infection, is incomplete. To probe pDC antiviral and immunomodulatory functions, we developed a model system in which the IFN-I response is pDC-restricted. We demonstrated that sensing of DENV and CHIKV infected cells by pDCs results in IFN-I production in the absence of other inflammatory cytokine responses. This pDC response leads to early control of both viruses and is sufficient to protect mice from CHIKV-induced lethality. Early pDC activation also results in an accelerated type II IFN response, via the activation of natural killer (NK) cells. Collectively, IRF7-mediated pDC activation orchestrates IFN-I and II responses controlling arbovirus infections.

HIV-1 and host restriction factors: relationship between viral reservoir and RFs expression levels

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Restriction Factors (RFs) are dominant proteins, belonging to innate immune system that target different essential steps of the HIV-1 life cycle, thereby providing an early line of defense against the virus. In this project we focused on the analysis of the expression levels of some of these cellular proteins, such as APOBEC3G, MX2, SAMHD1, SERINC3 and IFI16 cellular sensor with the aim to determine whether the variations of RFs expression profile are associated to the dynamics of HIV-1 replication towards the onset of a reservoir and disease progression. The expression profile variations of cellular factors were measured on PBMCs isolated from anonymous healthy donor blood donations and from residual samples obtained from HIV infected patients attending the St. Orsola University Hospital for routine virological monitoring. Samples were divided in three groups according to viral replication and treatment status: healthy donors, untreated HIV patients with detectable viremia and virally suppressed treated HIV patients. RNA was extracted from PBMCs and levels of expression of RFs were assessed using real time PCR methods. Our preliminary results revealed how MX2 is upregulated in infected patients compared to healthy controls. Furthermore, despite the huge variability linked to biological diversity between each subject, MX2 results mainly expressed in untreated patients compared to treated patients, where viral load is undetectable. Since MX2 is a restriction factor which plays a pivotal role in the early phases of HIV-1 life cycle, its up-regulation seems to be associated with the high levels of viral replication, always detectable in untreated patients in comparison with treated patients, where therapy is able to drastically reduce the number of infectious particles, but cannot effectively reach the viral reservoir. Our data support the notion that expression levels of RFs might have a role in determining HIV-1 replication and viral set point. However our aim will be to better understand the mechanisms which might influence the reservoir size and to analyze the role of viral proteins on RFs and the onset of latency.

Entry, Replication and Spread of Negative Strand Viruses: Lessons from Human Metapneumovirus

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Human metapneumovirus (HMPV) is a recently identified member of the pneumovirus family which causes significant morbidity and mortality worldwide. Work from our group and others suggest that many aspects of HMPV infection differ from those of previously characterized, closely related viruses. During viral entry, the HMPV fusion (F) protein is sufficient to promote attachment and entry on its own, with the help of the viral attachment protein, and entry involves binding to the attachment factor heparan sulfate and internalization of the virus. Recent work from our group has shown that efficient formation of viral replication centers, termed inclusion bodies, requires actin dynamics, and that inclusion body formation is a dynamic process. Finally, spread of respiratory viruses, including those of the paramyxovirus and pneumovirus families, generally involves assembly of individual viral particles which then are released and go on to infect new target cells. In contrast, we have recently shown that infection of human bronchial airway cells with HMPV results in formation of intercellular extensions and extensive networks of branched cell-associated filaments. Co-culture assays supported the hypothesis that viral spread from infected to new target cells occurs through these extensions, and viral proteins and viral RNA were detected in these structures, suggesting direct transfer of viral genetic material to new target cells. Overall, studies of HMPV have revealed novel mechanisms for entry, replication and viral spread.

Silenced or productive infection?
Engagement of an alphaherpesvirus with peripheral nervous system neurons

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Alpha herpesvirus infections stay life-long in infected human and animal hosts` nervous systems in a silent state ready to reactivate upon various stress signals. Remarkably, infection of epithelial cells with these viruses results in productive infection whereas infection of peripheral nervous system neurons results in non-productive silent infection (i.e. latency) in the natural hosts. More interestingly, infection of dissociated peripheral neurons in culture also results in productive infection unless DNA replication inhibitors are used. To study the molecular mechanisms of escape from latency, we used primary neurons cultured in compartmented tri-chambers. By this way, we recapitulated the natural route of infection by infecting axons with low dose of virus which resulted in a silent infection in a small number of neuronal cell bodies without the use of any inhibitors. Using these cultures, we developed a new complementation assay to investigate the molecular signals leading to escape from latency and establishment of productive infection. We found two different mechanisms to escape from silencing: a cellular stress-mediated slow route and viral tegument protein mediated-fast route. Furthermore, we showed that the stress-mediated pathway requires protein kinase A and c-Jun N-terminal kinase activity while the viral tegument-mediated fast escape does not require these host cell kinase activities. We also concluded that a general response to DNA virus infection or presence of excess herpesviral genomes in the nucleus to saturate silencing complexes is not enough to escape from silencing. Induction of a productive infection in neurons even at low MOI, requires either the presence of tegument proteins or the activation of the PKA and JNK pathway.

Influenza virus genome reaches the plasma membrane via a modified endoplasmic reticulum and Rab11-dependent vesicles

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The segmented negative-strand RNA genome influenza A viruses (IAVs) is replicated in the nucleus of infected cells. Neo-synthesized viral genomes, encapsidated into viral ribonucleoproteins (vRNPs), are exported from the nucleus and transported across the cytoplasm to the sites of viral budding at the plasma membrane. This process involves Rab11 GTPases but the precise mechanism remains poorly understood. We used metal-tagging and immunolabeling to visualise viral proteins and cellular endomembrane markers by electron microscopy of IAV-infected cells.

We provide evidence that IAV infection induces a major remodeling of the endoplasmic reticulum (ER) around the microtubule organizing center (MTOC) and all throughout the cell, as well as the formation of new organelles consisting of irregularly coated vesicles (ICVs). We demonstrate that vRNP components and the Rab11 protein are present on the modified ER and on ICVs, and that ICVs are distinct from recycling endosomes. Some ICVs are found very close to the ER and to the plasma membrane. ICV formation is observed only in infected cells and requires an active Rab11 GTPase.

Against the currently accepted model in which vRNPs are carried onto Rab11-positive recycling endosomes across the cytoplasm, our data strongly support a model in which (i) the modified ER is the first station of vRNPs after their exit from the nucleus, (ii) the ER is involved in the Rab11-dependent biogenesis of ICVs displaying Rab11 and vRNPs, (iii) ICVs then serve as the transport organelle for vRNPs from the ER to the plasma membrane.

Ex vivo modeling of Human Cytomegalovirus nasal entry site

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Human cytomegalovirus (HCMV) is a leading cause of congenital infection and neurosensorial disease of infectious origin, and a major pathogen in immunocompromised individuals. Horizontal HCMV transmission is known to occur following close contact with virus-shedding bodily excretions, yet the initial events of infection at the viral entry site, which could be an important intervention target, have remained ill defined. HCMV is a human specific virus, precluding animal modeling of early infection. While it has been assumed that HCMV enters new hosts via the oral route, combined epidemiological and murine-CMV experimental evidence suggest that HCMV infection may be initiated via the nasal route. Here we employed a novel human nasal turbinate organ culture for the *ex vivo* modeling of HCMV entry via the nasal mucosa. Fresh nasal turbinate tissues obtained during surgical procedures were maintained as integral multi-cell-type organ cultures, and their viability and natural histology were shown to be preserved for at least 7 days. Active viral replication and spread were observed following *ex vivo* infection of the nasal turbinate tissues, with the appearance of typical histopathological features of natural infection. HCMV infected a wide range of cells, exploiting a predominant cell-to-cell mode of spread within the nasal turbinate tissues. Employing a global transcriptome analysis, we further demonstrated a distinct innate immune response of the nasal turbinate tissues to HCMV infection. The *ex vivo* infected nasal turbinate model provides a unique insight into patterns of infection and innate tissue response during HCMV entry into the human host, and could further serve to evaluate the effects of new intervention strategies against the horizontal transmission of HCMV.

Dissection of influenza infection *in vivo* by single-cell RNA sequencing

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SUMMARY

The influenza virus is a major cause of morbidity and mortality worldwide. Yet, both the impact of intracellular viral replication and the variation in host response across different cell types remain uncharacterized. Here we used single-cell RNA-sequencing to investigate the heterogeneity in the response of lung tissue cells to *in vivo* influenza infection. Analysis of viral and host transcriptomes in the same single cell enabled us to resolve the cellular heterogeneity of bystander (exposed but uninfected) as compared to infected cells. We reveal that all major immune and non-immune cell types manifest substantial fractions of infected cells, albeit at low viral transcriptome loads relative to epithelial cells. We show that all cell types respond primarily with a robust generic transcriptional response, and we demonstrate novel markers specific for influenza-infected as opposed to bystander cells. These findings open new avenues for targeted therapy aimed exclusively at infected cells.

Note: This work has been recently accepted for publication:
Dissection of influenza infection in vivo by single-cell RNA sequencing, Yael Steuerman, Merav Cohen, Naama Peshes-Yaloz, Liran Valadarsky, Ofir Cohn, Eyal David, Amit Frishberg, Lior Mayo, Eran Bacharach, Ido Amit and Irit Gat-Viks. *Cell Systems*, in press.

Towards the identification and characterization of host factors in Adenovirus entry

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Human Adenoviruses (HAdVs) are widely used for the development of novel therapeutics based on cell specific gene delivery. This usage has been mainly derived from HAdVs high efficient cell transduction, as well as to its extensively characterized replication cycle. The virions enter cells by receptor-mediated endocytosis, activate lysosomal secretion, alter the lipid contents of the cell membrane, penetrate through the endosomal membrane and are transported to the nuclear pore complex where they release their genomes for translocation into the nucleus. Yet, many of the cellular factors involved in this remarkably efficient entry program are still unknown.

Here, we propose to use a proximity ligation-based proteomics approach to capture and define distinct steps during HAdV entry. To this end, we have generated a recombinant HAdV-C5 with an engineered ascorbate peroxidase (APEX2) fused to protein IX, a cement protein located on the outside of the virion. The IX-APEX2 fusion protein was incorporated into the viral capsid, and maintained the functionality of both protein IX and APEX2. We expect that this approach will resolve spatio-temporal virus-host interaction networks during entry, and give insight into the molecular ecology in the virion proximity.

High content screening/imaging of differentiated human primary neural cells identifies drug candidates that inhibits TBEV infection

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Tick-borne encephalitis virus (TBEV) is a leading cause of human neuro-infection in Europe and North-Eastern Asia. Despite its high medical relevance, no specific antiviral therapy is currently available. Development of relevant *in vitro* infection models and discovery of new broad-spectrum antiviral agents are needed to address this unmet demand. We describe a cell-based, high-content approach using an *in vitro* model consisting of primary neural cells differentiated from human foetal neural progenitor cells (hNPCs) for discovery of anti-TBEV compounds. The mixed culture constituted of neurons, astrocytes and oligodendrocytes represent a highly relevant and predictive cellular system for the identification of antiviral molecules active against neurotropic viruses. We screened a library of 100 compounds for their ability to block TBEV infection in our model. We monitored the effect of compounds by quantification of infected cells immune-stained with an antibody targeting the envelope protein of the virus, while simultaneously evaluating cytotoxicity. Established anti-flaviviral drugs and others that had no previously known antiviral activity were identified as inhibitors of TBEV infection. Several drugs previously identified on cell lines as anti-flaviviral candidates, were cytotoxic and did not reduce TBEV infection in our model. In addition, thanks to the high content analyses, we identified and quantified, based on DAPI staining, the neurons and astrocytes. Thus, we were able to identify what cells were targeted by TBEV and discriminate the action of drugs on these two cell populations. Our findings demonstrate that neural cells differentiated from hNPCs provide a cellular model exploitable in the search for active anti-flaviviral molecules.

Title: Analysis of Influenza A virus transcription at the single cell level

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Abstract: Influenza A virus (Orthomyxoviridae) is a negative stranded RNA virus with segmented genome that replicates in the nucleus of infected cells. During infection, Influenza A virus is highly dependent on interactions with the host RNA polymerase II. One of this interactions occurs during transcription where the virus RNA-dependent RNA-polymerase complex (RdRP) steals the 5' cap of host transcripts to primer viral mRNA expression. Elucidation of the specifics of these interactions is important to understand the impact of virus disruption on host transcription. We previously generated bulk RNA-seq data to simultaneously map cellular:viral RNA hybrids resulting from cap-snatching. We identified a selective cap-snatching bias resulting in avoidance of host protein transcripts required for viral replication. In recent years, single cell RNA-seq analysis of different tissues and cultured cell-lines has demonstrated that response to stimuli, such as virus infection, is largely heterogeneous. Here we generated single nuclei 5' expression libraries from uninfected and infected A549 lung-epithelial cells at high MOI [A/PR/8/1934(H1N1)] to analyze virus and host transcription at single cell resolution. Even at high levels of infection we detect heterogeneous levels of viral and host mRNA – including immune response– across infected cells, suggesting differences in virus susceptibility due to differences in cell-states. In agreement with our bulk RNA-seq data we detect biases on cap-snatching events. Characterization of these events and overall gene expression at single cell resolution will allow us to resolve whether such biases correlate with differences in cell-states, immune response and virus infection.

Mechanisms in cell-to-cell variability of virus infection

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Viruses have a dual nature, the particles (virions) and the viruses (infected cells). Virions are largely inert as long as they are extracellular. They change their nature and become dynamic when they contact a host cell at the onset of an infection. The processes and the outcome of a virus infection are variable between cells. This is due to heterogeneity in receptor abundance, endosomal and cytoplasmic trafficking, virion uncoating, the transcription of viral genes, the production and stability of viral proteins, the assembly of progeny particles from preformed components, and the egress of virions from the infected cell. All these steps are embedded into host and viral networks of proteins, nucleic acids, lipids, metal ions and metabolites. They involve innate immunity and stress response pathways with pro- and anti-viral effects. The nature of these networks and their impact on virus infection and viral residence in cells are key for the outcome of infection, as shown by single cell analyses of entry and gene delivery of DNA viruses into epithelial and immune cells (1-6). Here, I will discuss the heterogeneity in viral transcription, and elute to our earlier results demonstrating that the chemical activation of the unfolded protein response (UPR) boosted the transcription of the immediate early adenoviral transactivator protein E1A (7, 8). Maintaining and boosting E1A expression is key for viral persistence, and is mediated through enhanced levels of the host transcription factor XBP1. XBP1 controls the canonical UPR following ER stress and the activation of the inositol-requiring enzyme (Ire) 1 in the ER membrane. The combination of single cell / single virion analyses opens the door for deep investigations of mechanisms underlying cell-to-cell heterogeneity in viral infection.

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Molecular crosstalk between autophagy and Rubella virus

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Autophagy is a key homeostatic process in eukaryotic cells that results in lysosomal degradation and recycling of unnecessary or dysfunctional cellular components. However, some viruses can subvert this process to their own benefit interacting with the host autophagy machinery to control their replication and spread.

Rubella virus (RV) infection is generally known as mild symptomatic outcome in individuals, while congenital RV infection may lead to serious developmental abnormalities, collectively known as the congenital rubella syndrome, probably due to the RV interference with the host cell life cycle as the main cause of teratogenicity.

Therefore, we analysed the effect of RV infection on autophagy in human alveolar epithelial cells A549. Cells were infected with the wild type and lab-attenuated strain, C-77w and C-77a, respectively, with a multiplicity of infection of 1.0, in parallel, the expression level of genes encoding Beclin1, Atg5, Rab7, and p62(SQSTM1) proteins participating in different steps of autolysosome formation was measured.

The significant increase in *Beclin1* and *Atg5* gene expression at 24-48 (for the wild type) and 24-72 (for the attenuated type) hours after infection was observed, while significant induction of either *Rab7* or *SQSTM1* gene expression was not noticed. This effect was correlated with more delayed 2,5- to 5-fold increase of IFNβ-mediated *TRAIL* and *XAF1* gene expression leading to apoptotic cell death 72-96 hours after infection. Thus, we hypothesized that the autophagy process was abortive and did not lead to the virus elimination, while RV sustained viral growth through manipulating the apoptosis.

To investigate the role of autophagy on RV replication cycle, we measured the amount of infectious RV particles, together with the viral RNA in supernatants and cell lysates, after incubation of A549 cells with wild type or attenuated strain in the presence of the autophagy inhibitor, 10 nM Bafilomycin A1, or inducer, 100 nM Rapamycin. These experiments showed Bafilomycin A1 diminishing the RV infection non significantly, as evidenced by the RT-qPCR and plaque assay, while Rapamycin increased the amount of infectious RV particles released by the infected cells more dramatically with wild type comparing with attenuated strain.

Thus, we hypothesized that RV can use an antiviral mechanism to prevent degradation and ensure its replication, differentially regulating the process of autophagy, by stimulating the initiation and suppression of later steps.

Elucidating the relationship between molecular structure and antiviral activity of cationic amphiphilic drugs

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Enveloped virus families *Filovidirae*, *Arenaviridae*, *Rhabdoviridae*, *Coronaviridae*, *Togaviridae*, *Flaviviridae* and *Bunyaviridae* enter host cells via endosomal trafficking and low pH-dependent membrane fusion. Lack of effective antiviral treatment or vaccination makes novel antiviral drug development necessary. Cationic amphiphilic drugs (CADs) have broad antiviral properties but evoke also concerns about drug-induced phospholipidosis (DIPL) in host cells. Nevertheless, their antiviral mechanism of action and structure-function relationship is yet unclear. Therefore, we first aim to assess and compare the antiviral activities and cytotoxicity of CADs in a single-point approach and analyze which structural determinants enhance antiviral potential.

Due to CAD-sensitivity and high titer, MARV lentiviral particles were determined as best-suited pseudovirion. For the prototypical CAD amiodarone, IC_{50} = 1.8 µM and CC_{50} = 48.2 µM were determined in human endothelium/lung hybrid cells (EA.hy 926). We chose 5 µM as an optimal concentration to apply to all CADs and established a single-point assay with the conditions defined beforehand. Single-point testing of a panel of 47 CADs with various physico-chemical properties revealed clear differences between the CAD's ability to reduce viral transduction. The strongest antiviral activities were achieved with Dronedarone, Triparanol and Quinacrine with residual viral entry of 16 %, 18 % and 23 %, respectively. Simultaneously, the CAD's cytotoxicity was determined. Correlation analysis with the CAD's physico-chemical properties showed a positive correlation between antiviral activity and hydrophobicity, indicating logP>4 for strong antiviral CADs. Furthermore, first analyses showed that antiviral activity of CADs is associated with induction of DIPL.

In order to underline our findings, similar compounds to strong CADs will be identified and tested in several approaches. Moreover, by examining the antiviral range of potent CADs and their way of action, we will get more insight in the infection process of enveloped viruses.

Avian leukosis virus receptors and biotechnological approach to host resistance

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Avian sarcoma and leukosis virus (ASLV) diversified into six phylogenetically relative subgroups (A, B, C, D, E, and J) present as either exogenous or endogenous viruses in domestic chicken. These subgroups are unequivocally classified by the subgroup-specific receptor usage. ALV subgroups enter the cell through Tva, a protein belonging to the family of low-density lipoprotein receptors, Tvb, a tumor necrosis factor receptor-related protein, Tvc, a protein of the butyrophilin family with two immunoglobulin-like domains, or Tvj identified as the chicken Na+/H+ exchanger type 1 (chNHE1) with twelve predicted transmembrane segments and prominent extracellular loop 1. For all ASLV receptors, virus-resistant alleles exist, mostly due to the frame shift mutations or amino-acid substitutions. For example, single W38 deletion or substitution makes the NHE1 receptor molecule resistant to virus entry. Some of ASLV resistant receptor alleles segregate in domestic chicken and can be used for breeding the ASLV-resistant lines. On the other hand, resistant alleles for NHE1 have not been found in chicken.
In addition, we describe a new technique of transgenesis in chicken based on the primordial germinal cells (PGC). Orthotopic PGC transplantation into adult recipients and improved efficiency of gene modification (including gene introduction, CRISPR/Cas-9-mediated knock-outs and knock-ins) enable to skip the chimeric G_0 stage. This technique might become the state-of-art for biotechnological creation of ALV-resistance in the future.

Keywords: Avian leukosis virus, host resistance, transgenesis in chicken, receptor

Population heterogeneity of Varicella-zoster virus with different passages in cell culture

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Varicella-zoster virus (VZV) causes varicella and zoster, which can be prevented by live attenuated vaccines. Previous studies identified several nucleotide positions in the genomes of clinical and vaccine preparation that are genetically heterogenous, containing both wild-type and mutant (or vaccine-type) alleles. Thus, the VZV populations appear to be genetically heterogenous. Genetic heterogeneities of VZV populations with history of different passages *in vitro* cell culture were analyzed and compared. Genetic heterogeneity of VZV strain pOka prepared from pOka-BAC increased as the virus was propagated in cell culture to high passage. Both the number of genetically heterogenous sites (GHS) and the minor allele frequency (MAF) at GHS were significantly higher in high passage (p60) than in low passage (p3) populations of VZV. On the other hands, GHS in non-coding region decreased in high-passage strain. Genetic heterogeneity was mainly due to A to G and T to C transition events, thus increasing the G+C content in high-passage VZV population. Further analyses on the genetic heterogeneity in VZV populations will help to understand the molecular evolution of VZV propagated *in vitro* cell culture.

The intimate relationship between the UPR and innate immunity following Flavivirus infection

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Flaviviruses comprise an important virus family whose members are responsible for a myriad of epidemics in various parts of the world. Movement, globalization and climate change perpetuate their spread making control very crucial. The understanding of the host-virus interactions is therefore cornerstone in the attempts to produce efficient prevention and/or therapies against flaviviruses. The host immune system plays a critical role in fighting infections but viruses have evolved to deploy evasion strategies against the immune system thus abrogating the antiviral response that would otherwise function to clear the virus.

Tick-borne encephalitis virus (TBEV) has been described to efficiently delay the Interferon response during infection. A transcriptome analysis of TBEV infected cells during this delay revealed that additional to of the Interferon and Interferon stimulated genes (ISGs), there was an up-regulation of genes of the unfolded protein response (UPR) that were induced prior to interferon. Furthermore, when UPR was activated by external stimuli, the Interferon response including several ISGs, was induced much earlier through the IRF3 pathway leading to an antiviral effect. This antiviral response was dependent on the IRE1 arm of the unfolded protein response pathway, but was independent of the canonical interferon signaling. These observations were extended to other members of the Flavivirus family such as West Nile virus, Dengue virus and Zika virus. These data demonstrate the role and more so the kinetics of early innate signaling and how it is potentiated by the UPR in eliciting a potent antiviral response against flaviviruses.

Takeover by rabies virus G protein of signaling pathways driving neuron survival: a source of innovative therapeutical molecules for degenerative diseases

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Rabies virus is a strictly neurotropic virus that slowly propagates in the nervous system of the infected host from the site of entry (usually due to a bite) up to the site of exit (salivary glands). Successful achievement of the virus cycle relies on the preservation of the neuronal network. Once the rabies virus has entered the nervous system, its progression is not interrupted either by destruction of the infected neurons or by the immune response, which are the two major host mechanisms for combating viral infection. Rabies virus has developed two main mechanisms to escape the host defences: 1) its ability to kill protective migrating T cells and 2) its ability to sneak into the NS without triggering apoptosis of the infected neurons and preserving the integrity of neurites. The capacity of RABV to promote neuronal survival depends upon the virus envelope G protein capacity to recruit critical cellular partners. Following a virus-driven drug discovery we isolated from the rabies virus envelope G protein a new compound, called Neurovita, showing spectacular neuroprotective and neurodegenerative properties.

Borna disease virus utilizes IGF2BP2 for translational regulation

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Borna disease virus (BoDV), which belongs to the order *Mononegavirales*, establishes a persistent infection in the nucleus. Because BoDV replicates in the nucleus, the viral translation is separated from its transcription in space and time. This feature indicates that BoDV may control the quality and quantity of translating mRNAs to suppress virus production and maintain the persistent infection in the nucleus. However, translational regulation of BoDV has not been understood in detail.

To elucidate it, we performed screening of shRNA library using BoDV-infected cells and identified insulin-like growth factor 2 (IGF2) as a host factor associated with viral particle production. Knockdown of IGF2 enhanced translation activity and particle production of BoDV, and overexpression of IGF2 in IGF2-knockdown cells reduced BoDV translation. On the other hand, treatment of recombinant IGF2 protein to BoDV-infected cells had no effect on the viral production. These results suggest that not IGF2 protein, but its mRNA plays a role in the regulation of BoDV translation.

We therefore assessed the involvement of IGF2 mRNA binding proteins (IGF2BPs), which regulate IGF2 mRNA translation. Overexpression of IGF2BP2 enhanced the BoDV translation and particle production, whereas it decreased expression level of viral mRNAs in infected cells, like IGF2-knockdown. The RNA recognition motif and RhoGAP domains of IGF2BP2 were dispensable for its enhancing effect on viral translation. We also showed that IGF2BPs were co-immunoprecipitated with mRNAs and N protein of BoDV in infected cells. These results suggest that BoDV mRNAs undergo translational regulation of IGF2BP2 in competition with IGF2BPs-binding mRNAs, such as IGF2, for regulation of viral particle production in infected cells.

IL-15 modulates HIV resistance through SAMHD1 inactivation in CD4$^+$ T cells

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Antiretroviral treatment (HAART) inhibits productive HIV replication but fails to eradicate the virus because HIV integrates into the host genome. Memory CD4$^+$ T lymphocytes constitute a major component of this persistent HIV reservoir. Stimulation of CD4$^+$ memory T lymphocytes with common γc-chain cytokines such as IL-15 renders these cells temporarily more susceptible to HIV infection. IL-15 is up-regulated during primary HIV infection, a time when the HIV reservoir established. So, we investigated the molecular and cellular impact of IL-15 on CD4+T cell infection. We found that IL-15 stimulation induces SAMHD1 phosphorylation, which relieved an early block to infection. Analysis of the pathways downstream IL-15 receptor (IL-15R) revealed that SAMHD1 phosphorylation after IL-15 stimulation is JAK dependent. Indeed, treatment of CD4$^+$ T cells with Ruxolitinib, an inhibitor of the Janus kinases JAK1 and JAK2, effectively blocked IL-15 induced SAMHD1 phosphorylation thereby protecting CD4$^+$ T cells from HIV infection.

High-resolution single cell immune profiling using Mass Cytometry by time of Flight (CyTOF) showed that IL-15 stimulation altered the composition of CD4$^+$ T cell memory populations by increasing proliferation of memory CD4$^+$ T including the CD4$^+$ T stem memory cells (CD4$^+$ T_{SCM}) population. Importantly, a greater proportion of CD4$^+$ T_{SCM} expressed the phosphorylated form of SAMHD1 compared to the other CD4$^+$ T cell populations.

Taken together, CD4$^+$ T_{SCM}, a long-lived and self-renewing subset of T memory cells, are particularly vulnerable to HIV infection in the presence of elevated IL-15, a condition, which is found in clinical settings such as primary HIV infection. We propose that IL-15 plays a pivotal role in creating a self-renewing persistent HIV reservoir by facilitating infection of CD4$^+$ T cells with stem cell like properties. Time-limited interventions with JAK1 inhibitors such as Ruxolitinib could restore the ability of the endogenous restriction factor SAMHD1 to protect this long-lived CD4$^+$ T memory cell population from HIV infection.

Safety and immuno-efficacy profiles of the Yellow Fever vaccine versus wild-type infection in the Cynomolgus macaques (*Macaca fascicularis)*

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Yellow Fever virus (YFV) infection causes about 20% disease cases characterized by hemorrhages and multi-organ failures. In the 1930s the 17D live-attenuated vaccine was developed empirically by passaging the wild-type (wt) Asibi YFV strain in mouse and chicken embryo tissues. The 17D live-attenuated vaccine stands as a 'gold standard' for successful vaccination against YF, but the molecular determinants associated with its virulence attenuation and performance are still poorly understood. To better understand the pathogenic mechanisms of YF disease and to evaluate the safety and immuno-efficacy of the vaccine, we have used a non-human primate (NHP) model.

Post-vaccination and post-infection responses were studied in NHPs receiving one single Stamaril dose or 3 $LogCCID_{50}$ of Asibi strain, respectively, using an approach combining transcriptomics, safety/pathogenicity and immunogenicity analyses. Additionally, vaccinated NHPs received a virulent Asibi challenge 7 months after vaccination.

Asibi infection induced a virulent disease characterized by severe fever and clinical symptoms; strong disturbance of blood biochemistry, hematology, and cytokine parameters; and high viral load in blood and organs, leading to rapid death of all infected NHPs. As expected 17D vaccination resulted in a significant virulence attenuation, confirming its safety. The 17D vaccine induced strong and long-lasting neutralizing antibody titers; a sustained memory B cell response; and a moderate and transient IL-2 and IFNγ T cell response in peripheral blood, associated with protection against a subsequent virulent challenge with wt Asibi. Transcriptomics analysis demonstrated that, although both the wt strain and 17D vaccine induced the deregulation of similar gene sets (e.g. IFN-related pathways), there were more deregulated genes with a higher fold-change in Asibi infected NHPs.

The NHP model appears to be a helpful tool to decipher the mechanism of action of the 17D vaccine and to identify candidate biomarkers associated to vaccine safety and immuno-protection. Its translatability to humans will be further assessed following comparison with data from 17D vaccinated adult subjects (NCT01765413).

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CD81 positive exosomes play a pivotal role in establishment of persistent infection in chronic HCV patients

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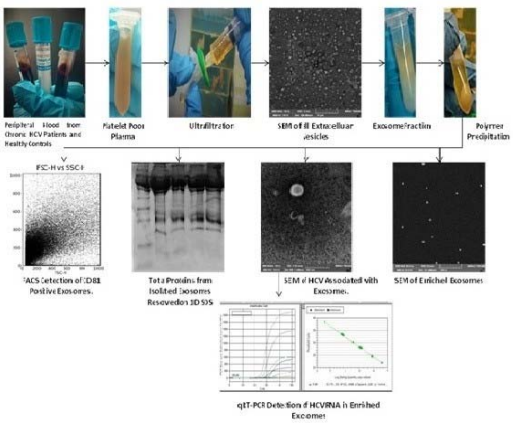
Abstract Body: Tetraspanin proteins, including CD81, are enriched in exosomes. We have enriched and isolated exosomes from peripheral blood of chronic hepatitis C (HCV) patients at all stages of liver disease. Exosomes were also enriched from peripheral blood of healthy individuals, who exhibited normal liver function test profile and had no known infection. The exosomes were first enriched by polymer precipitation accompanied with ultrafiltration. Enrichment of exosomes was then followed by total protein extraction and analysis via 1D SDS gel electrophoresis. Once presence of CD81 was roughly determined in the gel, immunoprecipitation of CD81 positive using dynabeads coupled with anti-CD81 antibody was performed. Isolated CD81 positive exosomes were then detented by anti-CD81 FITC coupled antibody using flow cytometery. HCV RNA was also detected in fractions of exosomes from HCV positive patients only via real time quantitative PCR. This confirmed the presence of CD81 positive exosomes and HCV RNA in same pool of enriched exosomes. We have also found that HCV particles were in associated with isolated CD81 exosomes either via surface interaction or as cargo of exosomes in electron micrograph. Therefore, we concluded that CD81 positive exosomes play a pivotal role in establishing persistent infection via immune evasion and possibility of extrahepatic infection.

Keywords: CD81, exosomes, HCV, immune evasion, persistent infection.



Towards understanding the role of sphingolipids and the unfolded protein response in lytic virus egress from Adenovirus-infected cells

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Human adenoviruses (HAdVs) are non-enveloped DNA viruses that cause life-threatening disease in immunocompromised people. Genetically engineered HAdV vectors selectively infect and kill malignant cells, and are widely used in human gene therapy trials as oncolytic agents. Over the past years the complexity and functionality of the lipidome in infected and uninfected cells have been unveiled, highlighting critical roles of bioactive sphingolipids in major cell signalling pathways. During HAdV entry sphingolipids play a critical role in membrane penetration. Enhanced levels of ceramide sphingolipids boost virus penetration through endosomes [1]. At late stages of infection, ceramide levels increase dramatically, yet the underlying mechanisms and impact on infection are largely unexplored [2]. Besides the lipid homeostasis also protein homeostasis is affected in the course of AdV infection. For example, chemical induction of the unfolded protein response (UPR) enhances AdV infection and spreading [3]. The UPR comprises a signalling network that orchestrates the recovery of ER function, or leads to apoptosis [4]. Here, we address if the induction of lipid or protein stress responses triggers persistently infected cells to enter the lytic phase towards virus spreading. We focus on the role of sphingolipids and explore their connection with the UPR in triggering lysis of HAdV-infected cells.

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Cellular localization of the proteins of the region 3 of feline enteric Coronavirus

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Feline enteric coronavirus (FECV) infection is very common in domestic cats all around the world. Approximately 20 to 60% of cats show seropositivity to the virus. As estimated, in around 5% of FECV infected animals, a progressive debilitating disease, feline infectious peritonitis (FIP) develops.

FECVs have three ORFs in region 3 (3a, 3b, and 3c). Many comparative diagnostic and experimental evidence implicate ORF3abc in the pathogenesis of FIP. In the case of FECV/ FIP virus (FIPV), the shifting of the focus of viral replication from the intestinal tract toward macrophages and monocytes usually goes together with deletions in the genomic regions 3. To gain additional data about the function of the proteins of FECV region 3, we expressed them as N-terminal eGFP fusion proteins in different cell lines and investigated them in silico with different prediction programs.

3a is predicted to contain DNA-binding and transcription activator domains, and it is localized in the nucleus and in the cytoplasm.

3b is also predicted to contain DNA-binding and activator domains, and was found to localize in the mitochondrion and in the nucleolus. Moreover, the accumulation of 3b was observed in a small percentage of infected and non-infected cells in the nuclear membrane.

3c of FECV is predicted to be a membrane protein and it is an ortholog (81% amino acid identity) of 3b of transmissible gastroenteritis coronavirus (TGEV). The exact compartmental localization of 3c remains yet to be determined. However, based on our co-localization studies, it is not localized in the intermediate-to-trans Golgi, ERGIC or the ER apparatus.

FIPV infection in CrFK cells had no visible effect on the localization of 3a and 3c from the plasmids. The expression of 3c-eGFP is clearly cell type-dependent, it is more stable in MARC 145 cells than in Fcwc-4 or CrFK cells, which might reflect in vivo stability differences in natural target cells (enterocytes vs monocytes/macrophages).

The SAT protein of Porcine Parvovirus accelerates ER stress and changes the localisation of the pro-apoptotic CHOP in the infected cells

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An ORF has been identified in porcine parvovirus (ungulate protoparvovirus-1, UPV-1), which overlaps with the VP2 ORF in an alternative frame and encodes the SAT protein (SATp). The SATp accumulates in the endoplasmic reticulum (ER) and in its absence a “slow spreading” phenotype develops in the attenuated NADL2 strain. The main objectives of our work were to investigate the effect of SATp in the pathogen Kresse strain and explore the cellular pathways through which SATp acts.

LDH activity measurement, propidium iodine, Hoechst staining, and attached cell count in infected PT cells revealed that the main form of cell death is lysis. At high multiplicity infection cell death correlated with virus release to the supernatant. Infection with SAT$^-$ virus resulted in reduced lysis and apoptosis in infected cells, and prolonged cell integrity slowed down viral release.

During UPV-1 infection the ER is gradually condensed perinuclearly, and regardless of the presence or absence of SATp, the expression of Xbp1 and CHOP was induced. Significant differences were detected in the quantity and localization of irreversible ER stress marker CHOP in SAT$^-$ and wild type virus-infected cells. Activation of CHOP by MG132 or DTT accelerated the egress and spreading of both the wild type and the SAT$^-$ viruses.

SATp alone does not activate the expression of the Xbp1 or CHOP but causes morphological changes in the ER similar to infection. CHOP expression in SAT$^-$ virus-infected cells does not result in accelerated spreading.

In conclusion, UPV-1 infection triggers ER stress both in the absence or presence of SATp. However, the presence of SATp makes the ER stress irreversible as it is indicated by CHOP expression. Chemically induced irreversible ER stress can fully compensate the loss of SATp confirming that the activation of irreversible ER stress response is indeed a necessary step to accelerate viral egress and spreading during UPV-1 infection.

Unravelling HIV-1 latency, one cell at a time

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An infection with the human immunodeficiency virus type 1 (HIV-1) can be treated but it cannot be cured, because of the extraordinary ability of the virus to integrate permanently into the genome of its host cells, resulting in some infected cells persisting as a latent reservoir. Single-cell omics offers new opportunities to answer important questions, for instance, Do latently HIV-1-infected CD4+ T cells have signature omics profiles that could be used for their identification? Can single-cell omics profiles define intracellular and extracellular factors and their interplay that might affect the reactivation of latent HIV-1 proviruses? Can single-cell omics profiles be used to predict outcomes of HIV cure interventions? Single-cell omics studies could define specific signature interactions between biomolecules (DNA, RNA, and/or protein) that govern HIV-1 latency, contributing towards curative efforts.

Densovirus experimental evolution *in insecta*: the appearance can be deceptive

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To understand the mechanisms underlying the evolution of insect parvoviruses and their adaptation to new hosts, we set up an experimental evolution protocol with an orally infectious densovirus pathogenic for several caterpillar species. Initiating viral populations from an infectious clone (a single genotype), we realised serial oral infections in parallel lines of two caterpillar species, either passaging the virus in the same species (constant environment) or alternating the host species (variable environment). After ten serial passages, the resulting phenotypes (virulence) and genotypes (viral genome sequences) were compared to the ancestral ones. Results showed that virulence dropped in constant environment and remained similarly high when alternating host species, suggesting that mutations were differentially selected with passages depending on the environment. The sequencing of the viral populations showed that, although different genomic variations occurred depending on the species and the environment, no adaptive mutations were selected that could support the phenotypic outcomes of infections we observed. When changing the mode of transmission from oral to systemic by injecting the virus to caterpillars, we found that all viral populations displayed the same virulence, which points the capsids interaction with the gut as the step that limits infection by viruses passaged in constant environments. To understand the mechanism involved, we deciphered the capsid interaction with the gut barrier components and found that depending on host origin, capsids interact differentially with the chitinous matrix secreted by the midgut epithelium, which constitutes the frontline of defence against pathogens. Although the interactions of the densovirus capsids with this matrix are probably more specific than initially thought, its overcome can be influenced by specific host-derived factors that are induced by infection and remain associated with semi-purified viral inoculum; the nature of these factors and their association with the capsids remain are yet to be characterized. In conclusion, these results illustrate an original mechanism that influences densovirus horizontal transmission and adaptation to new hosts.

Keywords: Densovirinae, insects, midgut, Peritrophic Matrix, densovirus-gut interaction

The Identification of Post Translational Modifications on the Zika Virus Precursor Membrane Protein and Their Roles in Virus Infectivity

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Introduction: The Zika virus (ZIKV) is an emerging arthropod-borne flavivirus that has put millions of people across the globe at risk of getting infected with the virus. Recently, neurological complications such as Guillain–Barré syndrome and fetal microcephaly have been associated with the disease. This has prompted the need for further studies on the virus to find effective drug and vaccine targets as it is believed that the virus has undergone several recombinations over the past decades during its interaction with the human host. The release of the virus from the host cell require the precursor membrane (prM) and the envelop (E) proteins interaction. The mechanism of this interaction is not well understood and inhibition of this interaction would be a potential therapeutic target for effective ZIKV drugs and vaccines. This study was conducted to identify ubiquitination sites on the ZIKV prM protein that are vital for the interaction with the E protein and subsequent release of infectious virions from the cell.

Methodology/Results: The prM protein was cloned in a plasmid vector and transfected in 293T to identify ubiquitination activity. Lysine amino acid residues were identified on the prM protein and individually mutated to arginine amino acid using site directed mutagenesis overlapping PCR. The PCR product was cloned in a plasmid vector and then co-transfected in 293T cells. Protein expression of the cell lysates were checked on SDS-PAGE to identify specific ubiquitination sites on the mutated proteins. Ubiquitin inhibition assay was done on ZIKV wild type. 6 ubiquitination sites were identified after immunoprecipitation and moreover inhibition of ubiquitin blocked the release of infectious virions from the cell.

Conclusion: The results demonstrate that the interaction between prM and E proteins is ubiquitin dependent and this has an effect on the release of infectious virions from the cell and continuity of infection. Development of drugs and vaccines that would inhibit this interaction would be an important step in the control and eradication of ZIKV disease.

The HIV-1 Tat protein affects human CD4$^+$ T-cell programing and activation, and favors the differentiation of naïve CD4$^+$ T cells

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Background: Chronic immune activation is strongly associated to AIDS progression and may force naïve T cell recruitment into memory lymphocytes under differentiation, thus contributing to the depletion of the naïve CD4$^+$ T pool and to the premature immunosenescence observed during HIV infection. It has been shown that the HIV-1 Tat protein, which is released by infected cells and may enter in neighboring uninfected cells, contributes to the hyperactivation of CD8$^+$ T lymphocytes. We wondered whether soluble Tat may also induce CD4$^+$ T cell activation and affect naïve CD4$^+$ T lymphocyte dynamics.

Methods: Purified CD4 T cells were activated by T cell receptor engagement in the presence or absence of Tat. Cytokine production, surface phenotype and expression of transcription factors important for T-cell programing were measured. Purified naïve CD4 T cells were cultured in nonpolarizing conditions in the presence or absence of Tat and their proliferation and differentiation was evaluated.

Results: CD4$^+$ T cells activated in the presence of Tat showed a significant increased production of IL-2, IFN-gamma and TNF compared to T lymphocytes activated in the absence of Tat. Moreover, Tat significantly enhanced the expression of transcription factors important for Th1 responses and T cell differentiation, such as Tbet and Eomes. As these data indicate that Tat favors CD4$^+$ T cell activation, we next assessed whether Tat affect the naïve CD4$^+$ T cell compartment.

Purified naïve CD4$^+$ T cells cultured in non-polarizing conditions proliferated and differentiated mostly towards a central memory phenotype. The addition of Tat significantly enhanced their proliferation and loss of naïveness, favoring the

differentiation towards transitional memory and effector memory cells, which were barely detected after culture in non-polarizing conditions without Tat.

Conclusions: Our results indicate that Tat favors $CD4^+$ T cell activation and thus may contribute to the chronic immune activation observed during HIV infection. Moreover, Tat affects naïve T cell dynamics enhancing their proliferation and differentiation towards memory subtypes, thus favoring their depletion and possibly participating in premature immunosenescence. These data suggest that anti-Tat immune responses may be important in preventing disease progression.

Snapweed as a reservoir of *Cucumber mosaic virus* in the region of Kaniv Natural Reserve

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Cucumber mosaic virus (CMV) is known to have one of the widest host ranges among plant viruses, infecting over 100 plant families worldwide.

At the same time, it poses threat to cultivated plants leading to major crop losses. This fact raises concern regarding the virus preservation in weeds surrounding the fields.
We sampled 22 weed species growing in a proximity to the fields in order to determine the plants hosting CMV and serving as potential source of infection for crops.

The results of ELISA have indicated the presence of CMV in snapweed (*Impatience*) samples gathered in 2 different locations. The presence of CMV in the snapweed was verified by RT-PCR and by bioassay to *Chenopodium amaranticolor* plants. Thus, snapweed growing in the areas surrounding fields needs to be considered as a reservoir and potential source for CMV infection of cultivated plants.

Single cell approaches to study the immune system

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A recent technological innovation - single cell multi-omics combined with imaging technologies that track dynamics and spatial organization - is now inspiring a new vision of understanding the progression of human diseases, at cellular resolution and genomic breadth. My group is interested to the molecular characterization of human T lymphocytes and to the dissection of the regulatory networks, that shape T cells identity and functional plasticity, exploiting single cell multi omics approaches in the context of tumor immunology.

Modeling Rotavirus Infection and Maternal Immunity in Human Enteroids

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Rotavirus is the primary etiology of moderate to severe diarrhea in infants. Understanding infection and protective mechanisms in humans has been difficult due to the lack of relevant models. We have developed adult and pediatric enteroid monolayers derived from human intestinal biopsies, and characterized cellular morphology, physiological and biochemical features. Macrophages and neutrophils have been added to generate immune enteroid co-culture models. Enteroid monolayers are permissive to rotavirus infection and exhibit cytotoxicity features similar to those seen in humans. Using pediatric enteroids, we also examined immune modulatory benefits of human breast milk on the intestinal mucosa. This presentation will focus on rotavirus pathogenesis and the effects of breastmilk in human pediatric enteroids, and the use of this ex-vivo model for mechanistic studies of enterovirus infection and maternal immunity.

Marcela F. Pasetti, Ph.D., is a Professor in the Departments of Pediatrics and Microbiology and Immunology, and a faculty member of the Center for Vaccine Development and Global Health (CVD) at the University of Maryland School of Medicine. Her research focuses on the characterization of immune responses following infection and vaccination in animal models and in humans. Her main areas of interest are maternal-infant immunization and the induction of protective immunity during pregnancy and early in life. Dr. Pasetti also studies mucosal immunity, particularly the role of antibodies in protection against mucosal pathogens. Through several NIH- and foundation-supported projects, her group is investigating mechanisms by which maternal immunity prevents enteric infections in young infants, serological predictors of vaccine efficacy, the role of vaccine-induced antibodies in preventing infection against mucosal pathogens, immune responses induced by novel adjuvants in humans, and markers of immunity to vaccine-preventable disease in young children. In addition to directing a thriving basic research program, Dr. Pasetti oversees the Applied Immunology Section at the CVD, which develops, refines, and performs a variety of immunological assays to support human clinical studies.

Virus-host interactions at the single-cell level – CMV gene expression dynamics and the innate immune response

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A highly dynamic gene activation process begins upon entry of CMV genomes into the host cell nucleus, both on the viral and on the host-cell side. The timing and velocity of gene expression events follow distinct patterns, but are also subject to considerable cell-to-cell heterogeneity. Experimental models to assess the dynamics of these processes at single-cell levels – and thus understand the processes shaping viral gene expression during the lytic cycle – are limited.

We developed a novel approach to visualise and quantify both CMV gene expression and endothelial cell interferon (IFN)-β expression at the single-cell level with temporal resolution. Using live-cell imaging and single-cell quantification of reporter genes encoded by recombinant mouse CMVs and an endothelial host cell line allows us to detect distinct temporal gene expression profiles.

Tracking the dynamic expression of essential immediate early (ie), early (e) and late (l) viral genes via fluorescence protein reporters in living infected cells, we observe that progression through the virus life cycle is remarkably robust. This observation even extended to a faction of cells in which the virus managed to initiate gene expression despite the presence of type-I IFN. Importantly, our method allowed us to detect subtle but significant changes in the timing of the onset, the maximal expression, as well the velocity of their expression at distinct points upon infection elicited by IFN. Applying this technique, we found that the inhibiting effect of type-I IFN on viral gene expression is mediated by both delaying its onset and decreasing maximal rates. Surprisingly, the specific role of PML, an IFN-stimulated antiviral gene and integral component of nuclear bodies seems negligible in this scenario.

Paramyxoviruses, interferon and persistence; variations at the molecular, viral, cellular and organism levels influence the outcomes of infection

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Paramyxoviruses are a large family of single stranded, negative sense RNA viruses that cause a variety of serious illnesses in humans and animals, ranging from respiratory infections to diseases such as measles, mumps and encephalitis (e.g. Nipah virus). We have been investigating how these viruses interact with the interferon (IFN) system; how they trigger an IFN response, how, once induced, interferon stimulate gene (ISG) products inhibit virus replication, and how paramyxoviruses, at least partially, circumvent the IFN response. Examples will be presented which show that the way different paramyxoviruses, and different isolates of the same virus, interact with the IFN system, at the molecular, cellular and organism levels, strongly influences the outcome of infections. In addition, a model of how these viruses may establish persistent infections will be presented. We have shown, at the molecular level, that at late times post-infection parainfluenza virus type 5 (PIV5) virus transcription and replication can be repressed in either an IFN-dependent or IFN-independent manner, leading to the establishment of persistent infections in tissue culture cells. In persistently infected cultures PIV5 fluxes between active and repressed states within individual cells. As single amino acid substitutions in different PIV5 isolates determines whether or not virus replication can be repressed, we speculate that *in vivo,* during early acute phases of virus infection variants of PIV5 will be selected for in which virus replication will not be switched off as these viruses will have a selective replicative advantage. However, as the infection progresses, variants in which virus replication can be repressed will be selected as they may establish prolonged/persistent infections in the face of a developing adaptive immune response.

Single cell transcriptomics for characterizing the human immune response to yellow fever virus

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Systems immunology methods have demonstrated great utility for studying immune responses to different vaccines and pathogens. Transcriptomics techniques serve as core components of systems-level characterizations and are often applied to complex mixtures of immune cells such as peripheral blood mononuclear cells (PBMC). Such “mixed cell” transcriptional profiles present challenges in associating gene expression (and corresponding function) with specific cell types, and can also obscure functionally significant contributions by rare cells. Recently developed high throughput droplet microfluidics RNA-Seq strategies have the potential to overcome these issues. We applied the inDrops RNA-Seq method to characterize the human immune response to yellow fever vaccine (YFV) in PBMC at single cell resolution. Our analysis has revealed cell type-specific gene expression programs in both innate and adaptive components of the response to this highly effective and clinically important vaccine.

Inhibition of Chikungunya virus nsP2pro cysteine protease by structure-based peptidomimetics antivirals

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Chikungunya virus (CHIKV), a mosquito born pathogenic virus, is responsible for causing rashes, arthritis, encephalitis, and death in humans. CHIKV is a global public health threat and has caused several outbreaks around the world. Currently, no vaccines or specific drugs/antivirals are available against CHIKV infection. The replication machinery of CHIKV consists of four nonstructural proteins (nsP1–4), produced as a single polypeptide, processing of which is carried out by nsP2pro by cleaving it into individual nsPs. Therefore nsP2 constitutes a promising drug target. In the present study, crystal structure of CHIKV nsP2pro has been deduced and structure-based protease inhibitors are identified. CHIKV nsP2pro structure revealed that the protein is comprises of two subdomains (N-terminal protease and a C-terminal MTase-like subdomain) and active site of the protein, which is located at the interface of two subdomains, is gated by a flexible loop. Based on the crystal structure of CHIKV nsP2pro (PDB ID: 4ZTB), two peptidomimetic compounds are identified and further assessed by molecular docking and molecular dynamics simulation for structural stability and conformational flexibility. Additionally, a FRET based nsP2 protease activity assay is developed and protease inhibitory potential of the identified peptidomimetic compounds is established. Inhibition of CHIKV nsP2pro by the tested compounds PepI & PepII is observed with IC_{50} values of 34µM and 42µM, respectively. The inhibition kinetic studies showed that the inhibition constant (K_i) is 33.34±2.53 µM for PepI and 45.89±4.38 µM for PepII. Additionally, these two compounds are further validated by cell culture based plaque reduction assay and found to be significantly inhibiting CHIKV replication in BHK-21 cells at concentrations much lower than their cytotoxic concentrations. This approach of structure-based identification of peptidomimetics and evaluating their *in vitro* & *in vivo* efficacies for inhibiting CHIKV nsP2pro may further lead to novel antiviral drugs against chikungunya infection.

Monitoring, predicting and targeting virus evolution and transmission

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RNA viruses generate huge mutant swarms that allow rapid evolution within a host. While NGS technologies allow us to identify the thousands of mutants in a virus population, identifying which mutations and composition of variants is relevant to infection remains a challenge. We combined mathematical dimension reduction methods and new mathematical matrix algorithms to identify biological signals in NGS data to monitor RNA virus evolution. We show that despite the high theoretical dimensionality of sequence space, the biologically relevant sequence space is of low dimensionality and can track virus evolution. We reconstruct genotype-phenotype landscapes and show that minority variants contribute significantly to fitness, and allows for better prediction of phenotype. We illustrate how distribution-based modeling of sequence space time dynamics can help predict virus evolution and alter it in antiviral approaches.

The age of organoids: new ways for virus-host interaction studies

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Recent advances in human 3D tissue culturing have created the opportunity to study virus-host interactions in a human setting. Stem cell-derived organotypic models or organoids are *in vitro* 3D models that have been developed for multiple organs (*e.g.* intestine, lung, brain *etc.*) and closely recapitulate the *in vivo* situation in terms of heterogeneity and organization of cell types. Organoids could revolutionize virus research that currently relies heavily on 2D cell culture and animal models, which have limited potential for studying human-pathogen interaction. For instance, EV-A71 virus (considered to be the new poliovirus) can cause neurological disease in mice but in these animals it is not able to replicate in the primary entry sites (respiratory or gastrointestinal tract). In humans, EV-A71 has been associated with large outbreaks of hand-foot-mouth disease, which can lead to severe complications such as encephalitis or pneumonitis. Studies in mice and cynomolgus monkeys point towards a glutamic acid (E) at residue 145 of the VP1 capsid protein as a marker for increased (neuro)virulence. On the other hand, whole genome analyses of clinical isolates from humans associate glutamine (Q) at VP1-145 to more severe disease. This contrast in clinical and animal studies along with genomic diversity and strain adaptation to laboratory (and animal) culture severely hampers the understanding of EV-A71 pathogenesis in humans. Therefore, we developed human 3D cell culture models to study virus pathogenesis directly in a human setting. We tested EV-A71 replication in organoids derived from primary enterovirus entry sites *i.e.* gut and lung epithelia. Analysis by RT-qPCR and virus titration assay reveal strain specific replication kinetics in the gut and lung organoid models. Glutamine at VP1-145 of EV-A71 replicates more efficiently on both lung and gut organoids than glutamic acid at the same residue. These results are in concordance with analyses of clinical isolates mentioned earlier, and highlight the potential for 3D cell culture models to increase our understanding of virus pathogenesis. In conclusion, human 3D cell cultures are unique tools to study the natural microenvironment of human infections, providing a better alternative for the use of *in vitro* or animal models.

Exploring host-viral interactions during Mimivirus Infection using quantitative analyses

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During infection with mimivirus, amoeba cells transform from free-living protozoans to cells containing very large viral factory (VF), which represent elaborate organelles that exploit host factors to effectively assemble multiple viral progeny that are eventually released through host lysis. The time windows during which each of the fundamental events in mimivirus infection has been determined only roughly, mainly due to the regular use of non-statistical imaging techniques. Thus, accurate kinetic analysis of mimivirus infection is necessary to determine whether - and to what extent - host and virus factors or external perturbations impact specific processes in the infection cycle. Due to the heterogeneity of individual viral-cell interactions, accurate kinetics is best achieved by analyzing very large populations. We have therefore developed a high-throughput method for monitoring the progression of the mimivirus infection cycle by tracking key aspects of infected amoeba cells using Image Flow Cytometry (IFC). We found that the infection cycle can be divided into three distinct phases. Key events in the infection cycle can be ordered in relation to one another, and to these phases. We utilized this approach to explore viral-host interactions by measuring altered infection cycle kinetics derived from disruption of microtubules or actin microfilaments, accompanied by the use of super-resolution microscopy to reveal cytoskeleton rearrangement during infection. Our results imply that although both types of fibers are modified, the microtubule framework does not seem to be directly required for mimivirus infection, the disruption of actin microfilaments significantly affects the infection cycle, as the fusion of replication centers into a mature VF is inhibited, the production of new virions is decreased, and finally the overall viral yield is reduced.