EUROPEAN SEMINARS in VIROLOGY

9th European Seminar in Virology (EuSeV)

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

October 21-23, 2022

Next Gen Virology – New Frontiers and Methodologies in Research and Diagnostics

Abstract book and program

EUROPEAN SOCIETY for VIROLOGY

9th European Seminar in Virology (EuSeV)

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Next Gen Virology – New Frontiers and Methodologies in Research and Diagnostics

Organizers:

Gabriella Campadelli-Fiume,
University of Bologna

Dana Wolf,
Hebrew University Jerusalem

Thomas Stamminger,
Ulm University Medical Centre

on behalf of the European Society
for Virology (ESV)
President Michael Kann

9th European Seminars in Virology 2022: Next Gen Virology – New Frontiers and Methodologies in Research

Program

FRIDAY 21.10.2022	
16:00-16:15	**Welcome** **Gabriella Campadelli-Fiume, Dana Wolf, Thomas Stamminger, Michael Kann**
Cellular and viral structures **Chair: Michael Kann, Adam Grundhoff**	
16.15–16.45	Ileana Cristea *(Princeton University, Department of Molecular Biology, Princeton, USA) (virtual talk)* Dynamic organelle remodeling: Viral mechanisms to subvert host cell biology
16.45–17.15	Jens Bosse *(Hannover Med School, RESIST group Quantitative Virology, CSSB Ctr for Structural Systems Biology, c/o DESY, Hamburg, Germany)* Spatiotemporal orchestration of herpesvirus morphogenesis
17.15 - 17.30	**Myriam Scherer[1]**, Clarissa Read[1,2], Gregor Neusser[3], Christine Kranz[3], Anna K. Kuderna, Regina Müller[4], Florian Full[5], Sonja Wörz[1], Anna Reichel[6], Eva-Maria Schiling[1], Paul Walther[2], Thomas Stamminger[1] [1]Institute of Virology, Ulm University Medical Center, Ulm, Germany [2]Central Facility for Electron Microscopy, Ulm University, Ulm, Germany [3]Institute of Analytical and Bioanalytical Chemistry, Ulm University, Ulm, Germany [4]Institute of Clinical and Molecular Virology, Friedrich Alexander University Erlangen-Nuremberg, Erlangen, Germany [5]Institute of Virology, University Medical Center and Faculty of Medicine, Albert-Ludwig-University Freiburg, Freiburg, Germany [6]Institute for Molecular Medicine, Lisboa, Portugal Combined Interferon and DNA Damage Signaling Induces Giant PML Cages that Restrict HCMV by Entrapment of Viral Genomes and Capsids
17.30-17.45	Vesa Aho[1*], **Sami Salminen[1]**, Salla Mattola[1], Alka Gupta[1], Felix Flomm[2,3,4,5], Beate Sodeik[4,5], Jens B. Bosse[2,3,4,5] and Maija Vihinen-Ranta[1] [1]Department of Biological and Environmental Science, Nanoscience Center, University of Jyväskylä, Finland [2]HPI, Leibniz-Institute for Experimental Virology, Hamburg, Germany [3]Centre for Structural Systems Biology, Hamburg, Germany [4]Hannover Medical School, Institute of Virology, Hannover, Germany [5]Cluster of Excellence RESIST (EXC 2155), Hannover Medical School, Hannover, Germany Infection-induced chromatin modifications facilitate translocation of herpes simplex virus capsids to the inner nuclear membrane
17.45-18.00	Boris Bogdanow, Iris Gruska, Lars Mühlberg, Jonas Protze, Svea Hohensee, Barbara Vetter, Martin Lehmann, Lüder Wiebusch, **Fan Liu** Structural interactomics, Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), Berlin, Germany Spatial, Quantitative and Functional Deconstruction of Protein Interactions Inside Cytomegalovirus Particles
18.00-18.15	**Boris Bogdanow[1]**, Lars Mühlberg[1], Iris Gruska[2], Barbara Vetter[2], Arne Elofsson[3], Lüder Wiebusch[2], Fan Liu[1]

	[1]Research group „Structural Interactomics“, Leibniz Forschungsinstitut für Molekulare Pharmakologie, Berlin, Germany [2]Labor für Pädiatrische Molekularbiologie, Charité Universitätsmedizin Berlin, Berlin, Germany [3]Stockholm Bioinformatics Center, Stockholm University, SE-106 91, Stockholm, Sweden Herpesvirus-Host Protein Interaction Contact Site Profiling from Intact Infected Cells by AI-assisted Structural Proteomics
18.15-18.30	**Anna Luganini[1]**, Valentina Serra[1], Giorgia Scarpellino[2], Shree Madhu Bhat[1], Luca Munaron[1], Alessandra Fiorio Pla[2], and Giorgio Gribaudo[1] [1]Laboratory of Microbiology and Virology, [2]Laboratory of Molecular Angiogenesis, Department of Life Sciences and Systems Biology, University of Turin, Turin, Italy The US21 viroporin of Human Cytomegalovirus is a novel regulator of cell adhesion and migration

18.45-19.30 POSTER SESSION - DISCUSSIONS IN FRONT OF POSTERS

Josua Janowski[1,3], Petya Berger[2], Rexford Mawunyo Dumevi[2,4], Alexander Mellmann[2], Stephan Ludwig[1,5], Linda Brunotte[1,5]
[1]Institute of Virology Muenster, University of Muenster
[2]Institute of Hygiene Muenster, University of Muenster
[3]SP BioSciences Graduate Program, University of Muenster
[4]EvoPAD Research Training Group 2220, University of Muenster
[5]Interdisciplinary Center for Clinical Research, University of Muenster

Increased vRNA-dependent immune induction conferred by mutations PB1-R386K, PB1-A14V and PB2-N567D in the viral polymerase of avian influenza A viruses

Fawad Khan[1,2], Thomas R. Müller[3,4], Mario Alberto Ynga Durand[1,2], Bahram Kasmapour[1,2], Dirk H. Busch[3,4], Luka Cicin-Sain[1,2]
[1]Helmholtz Centre for Infection Research, Viral Immunology, Braunschweig, Germany
[2]German Centre for Infection Research, Hannover-Braunschweig, Germany
[3]Technical University of Munich, Institute for Medical Microbiology, Immunology and Hygiene, Munich, Germany
[4]German Centre for Infection Research, Munich, Germany

Early recognition of HCMV-pp65 antigen by CD8 T cells

Jens Kleinehr[1], Katharina Daniel[1], Franziska Günl[1], Josua Janowski[1], Linda Brunotte[1], Marie Liebmann[2], Matthias Behrens[3], Andrea Gerdemann[3], Luisa Klotz[2], Hans-Ulrich Humpf[3], Stephan Ludwig[1], Eike R. Hrincius[1]
[1]Institute of Virology Muenster (IVM), Westfaelische Wilhelms-University Muenster, Von-Esmarch-Strasse 56, 48149 Muenster, Germany
[2]Department of Neurology with Institute of Translational Neurology, University Hospital Muenster, Albert-Schweitzer-Campus 1, 48149 Muenster, Germany
[3]Institute of Food Chemistry, Westfaelische Wilhelms-University Muenster, Corrensstrasse 45, 48149 Muenster, Germany

Impact of glycolytic interference via 2-deoxy-D-glucose on the replication of influenza A virus

Rok Kogoj, Nataša Knap, Jan Slunečko, Patricija Pozvek, Misa Korva
Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Development and implementation of a semi-automated SARS-CoV-2 molecular detection workflow for meeting the demand of daily testing requirements

Luca Ruotolo[1], Silvia Silenzi[2], Beatrice Mola[1], Alessia Bertoldi[1], Tizziana Lazzarotto[1,2], Giada Rossini[2]
[1]Section of Microbiology, Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Italy.
[2]Microbiology Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Italy

Epidemiological monitoring of SARS-CoV-2 variants circulating in the metropolitan area of Bologna, April-September 2022	
Marianna Terreri, Beatrice Tosoni, Ilaria Frasson, Sara N. Richter University of Padua, Department of Molecular Medicine, Padua, Italy. HIV latency: the role of G-quadruplex structures in proviral transcription in latently infected cells	
Fakhar Waqas[1*], Frank Pessler[1,] [1]TWINCORE, Center for Experimental and Clinical Infection Research, Hannover, Germany [2]Medizinische Hochschule Hannover, Hannover, Germany Proviral effects of ISG15 and antiviral potential of NRF2 activators against human Coronavirus infection in IPSC-derived vascular endothelial cells and Calu-3 cells	
20:00	**Dinner at outside Restaurant**

SATURDAY 22.10.2022	
Gene expression and replication I **Chair: Thomas Stamminger, Jens Bosse**	
08.30-09.00	Adam Grundhoff *(Leibniz-Institute of Virology, Virus Genomics, Hamburg, Germany)* Chromatin programming in herpesvirus latency
09.00-09.15	Ido Lavi, Supriya Bhattacharya, Ola Orgil, Ankita Awase, Nir Avital, Guy Journo, Vyacheslav Gurevich and **Meir Shamay** Daniella Lee Casper Laboratory in Viral Oncology, Azrieli Faculty of Medicine, Bar-Ilan University, Safed, Israel Unidirectional recruitment is essential for viral latency
09.15-09.30	Alona Kuzmina, **Ran Taube** Ben-Gurion University, The Shraga Segal Microbiology, Immunology and Genetics, Beer Sheva, Israel Role of long non-coding RNAs (lncRNAs) in HIV latency
09.30-09.45	Ofir Cohn, Gal Yankovitz, Naama Peshes-Yaloz, Yael Steuerman, Amit Frishberg, Rachel Brandes, **Eran Bacharach, Irit Gat-Viks** The Shmunis School of Biomedicine and Cancer Research, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel Distinct gene programs underpinning 'disease tolerance' and 'resistance' in influenza virus infection
9.45-10.00	**Duygu Merve Çalışkan[1,2]**, Sriram Kumar[1,2], Klaus Schughart[3], Rainer Wiewrodt[4], Karsten Wiebe[5], Peter Barth[6], Alexander Mellmann[7], Stephan Ludwig[1,2], Linda Brunotte[1] [1]Institute of Virology, University of Münster, Germany [2]EvoPAD Research Training Group 2220, University of Münster, Germany [3]Department of Infection Genetics, Helmholtz Centre for Infection Research, Braunschweig, Germany [4]Department of Medicine A, Hematology, Oncology and Pneumology, University Hospital Münster, Germany [5]Department of Thoracic Surgery, University Hospital Münster, Germany [6]Gerhard-Domagk-Institute of Pathology, University of Münster, Germany, [7]Institute of Hygiene, University of Münster, Germany Characterizing the pathogenicity determinants of a clinical influenza B virus isolate from the 2017/18 influenza season

BREAK	
Gene expression and replication II Chair: Thomas Stamminger, Jens Bosse	
10.30-11.00	Noam Stern-Ginossar (*Weizmann Institute of Science, Department of Molecular Genetics, Rehovot, Israel)* mRNA translation – a tug of war during viral infection
11.00-11.15	**Anne-Charlotte Stilp[1]**, Myriam Scherer[1], Patrick König[1], Axel Fürstberger[2], Hans A. Kestler[2], Thomas Stamminger[1] [1]Institute of Virology, Ulm University Medical Center, Ulm, Germany [2]Institute of Medical Systems Biology, Ulm University, Ulm, Germany ATRX acts as a modulator of chromatin accessibility following interferon signaling
11.15-11.30	**Sriram Kumar[1]**, Saskia Sophie Hinse[1], Duygu Merve Caliskan[1], Angeles Mecate-Zambrano[1], Klaus Schughart[2], Alexander Mellmann[3], Joachim Kühn[1], Rainer Wiewrodt[4], Karsten Wiebe[5], Peter Barth[6], Stephan Ludwig[1], *Linda Brunotte[1] [1]Institute of Virology, University of Münster, Germany [2]EvoPAD Research Training Group 2220, University of Münster, Germany [3]Department of Infection Genetics, Helmholtz Centre for Infection Research, Braunschweig, Germany [4]Department of Medicine A, Hematology, Oncology and Pneumology, University Hospital Münster, Germany [5]Department of Thoracic Surgery, University Hospital Münster, Germany [6]Gerhard-Domagk-Institute of Pathology, University of Münster, Germany, [7]Institute of Hygiene, University of Münster, Germany Unravelling the IFN-α Subtype-Specific Transcriptional and Antiviral Responses to Exogenous Treatment in Native Human Lung Tissues
Systems Biology Approaches I **Chair: Gabriella Campadelli-Fiume, Nicole Fischer**	
11.30-12.00	Schelhaas, Mario *(Westfälische Wilhelms-Universität Münster, ZMBE, Institut für Zelluläre Virologie, Münster, Germany)* Systems biology approaches as leads to unravel the cell biology of papillomavirus entry
12.00-12.30	Ujjwal Neogi *(Karolinska Institutet, Division of Clinical Microbiology, Department of Laboratory Medicine, Huddinge, Sweden* Genome-scale metabolic atlas for emerging and re-emerging RNA viruses
LUNCH BREAK **(Meeting of the ESV EC/AB)**	
Systems Biology Approaches II **Chair: Gabriella Campadelli-Fiume, Nicole Fischer**	
14.00-14.30	Finn Grey *(University of Edinburgh, Roslin Institute, Easter Bush Campus, Midlothian, UK)* Identification of novel host-virus interactions using high throughput screens
14.30-15.00	Silke Stertz (*University of Zürich; Institute of Medical Virology, Zürich, Switzerland)* MHC class II as novel entry receptor of influenza A viruses
15.00-15.30	Christian Münch *(University Hospital Frankfurt, Goethe University, Institute of Biochemistry II, Frankfurt, Germany)* Host cell proteome dynamics during coronavirus infection

15.30-15.45	**Jan Knickmann[1]**, Laura Staliunaite[1], Olha Puhach[1], Eleonore Ostermann[1], Thomas Günther[1], Jenna Nichols[2], Adam Grundhoff[1], Michael Jarvis[3], Sebastian Voigt[4], Andrew Davison[2], Wolfram Brune[1] [1]Leibniz Institute of Virology, Hamburg, Germany [2]MRC-University of Glasgow Centre for Virus Research, Glasgow, UK [3]University of Plymouth, Plymouth, Devon, UK [4]Institute for Virology, University Hospital Essen, Essen, Germany STAR cloning – a new method for rapid cloning of complete herpesvirus genomes
BREAK	
Focus on SARS-CoV-2 **Chair: Dana Wolf, Jutte de Vries**	
16.15-16.45	Birgit Sawitzki *(Berlin Institute of Health (BIH) & Charité University Medicine, Translational Immunology, Campus Virchow-Klinikum, Berlin, Germany)* Unravelling mechanisms behind T cell pathology during severe COVID-19
16.45-17.15	Matteo Iannacone *(San Raffaele Scientific Institute & University, Dynamics of Immune Responses, Milan, Italy)* Immune responses to HBV and SARS-CoV-2
17.15-17.45	Jutte De Vries *(Leiden University Medical Center, Clinical Microbiological Laboratory, Leiden, The Netherlands)* State of the art in clinical viral metagenomics – advances and today's challenges
17.45-18.00	**Vibhu Prasad[1]***, Berati Cerikan[1], Yannick Stahl[1], Katja Kopp[1], Vera Magg[1], Charlotta Funaya[2], Uta Haselmann[1], Mirko Cortese[1,&], Florian Heigwer[3], Josephine Bageritz[3,†], David Bitto[4], Saruul Jargalsaikhan[1], Christopher Neufeldt[1,§], Felix Pahmeier[1,#], Michael Boutros[3], Yohei Yamauchi[4], Alessia Ruggieri[1], Ralf Bartenschlager[1,5,6,7]* [1]Department of Infectious Diseases, Molecular Virology, Heidelberg University, Heidelberg, Germany [2]Electron Microscopy Core Facility, Heidelberg University, Heidelberg, Germany [3]Division of Signaling and Functional Genomics, German Cancer Research Center, and Department of Cell and Molecular Biology, Heidelberg University, Medical Faculty Mannheim, Mannheim, Germany. [4]School of Cellular and Molecular Medicine, Faculty of Life Sciences, Biomedical Sciences Building, University of Bristol, Bristol, UK. [5]Division Virus-Associated Carcinogenesis, German Cancer Research Center, Heidelberg, Germany [6]German Center for Infection Research, Heidelberg Partner Site, Heidelberg, Germany [&] Present address : Telethon Institute of Genetics and Medicine, Pozzuoli, Italy [$] Present address : Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, USA [#] Present address : Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, Berkeley, CA, USA [†] Heidelberg University, Centre for Organismal Studies, Research group Stem Cell Niche Heterogeneity, Im Neuenheimer Feld 230, 69120 Heidelberg, Germany Enhanced SARS-CoV-2 entry via UPR-dependent AMPK-related kinase NUAK2
20:00	**Dinner at outside Restaurant (Bus transfer at 18.15)**

SUNDAY 23.10.2022	
Diagnostic and antiviral approaches **Chair: Michael Kann, Silke Stertz**	
09.00-09.30	Nicole Fischer *(University Medical Center Hamburg-Eppendorf, Medical Microbiology, Virology and Hygiene, Hamburg, Germany)* Application of pathogen metagenomics - before, during, and after COVID-19
09.30-10.00	Dana Wolf (*Hadassah University Hospital, Dept. of Clinical Microbiology & Infectious Diseases, Clinical Virology Unit, Jerusalem, Israel)* Proteomic discovery of new prenatal biomarkers which predict the severity of congenital CMV infection
10.00-10.15	Annika Rössler[1], Antonia Netzl[2], Ludwig Knabl[3], Helena Schäfer[1], Samuel H. Wilks[2], David Bante[1], Barbara Falkensammer[1], Wegene Borena[1], **Dorothee von Laer[1]**, Derek Smith[2], Janine Kimpel[1] [1]Institute of Virology, Department of Hygiene, Microbiology and Public Health, Medical University of Innsbruck, Innsbruck, Austria [2]University of Cambridge, Center for Pathogen Evolution, Department of Zoology, Cambridge, UK [3]Tyrolpath Obrist Brunhuber GmbH, Zams, Austria Multiple exposure to SARS-CoV-2 enhances cross-neutralizing antibodies to antigenically distinct, non-exposed variants
10.15-10.30	**Leila Abassi[1]**, Federico Bertoglio[2], Philip Alexander Heine[2], Thomas Schirrmann[3,4], Michael Hust[2], Luka Čičin-Šain[1,5] [1]Helmholtz Centre for Infection Research, Department of Viral Immunology, Inhoffenstr. 7, 38124 Braunschweig, Germany [2]Technische Universität Braunschweig, Institut für Biochemie, Biotechnologie und Bioinformatik, Abteilung Biotechnologie, Spielmannstr. 7, 38106 Braunschweig [3] YUMAB GmbH, Inhoffenstr. 7, 38124 Braunschweig, Germany [4]CORAT Therapeutics GmbH, Inhoffenstr. 7, 38124 Braunschweig, Germany [5]German Centre for Infection Research (DZIF), Hannover-Braunschweig site, Inhoffenstr. 7, 38124 Braunschweig, Germany Evaluation of neutralizing antibodies against SARS-CoV-2 infection
10.30-10.45	**Baxolele Mhlekude[1,2]**, Dylan Postmus[1,2], January Weiner 3rd[2], Saskia Stenzel[1,2], Francisco Zapatero[3,4,5], Ruth Olmer[6], Jenny Jansen[1,2], Anja Richter[1], Julian Heinze[1], Nicolas Heinemann[1], Barbara Mühlemann[1], Simon Schroeder[1], Terry C. Jones[1,7], Marcel Alexander Müller[1], Victor Max Corman[1], Christian Drosten[1], Andreas Pich[8], Volker Thiel[9,10], Ulrich Martin[6], Daniela Niemeyer[1], Gisa Gerold[3,4,5], Dieter Beule[2], Christine Goffinet[1,2] [1]Institute of Virology, Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, Charitéplatz 1, 10117 Berlin, Germany [2]Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Charitéplatz 1, 10117, Berlin, Germany [3]Department of Biochemistry, University of Veterinary Medicine Hannover, 30559 Hannover, Germany [4]Institute of Experimental Virology, TWINCORE, Centre for Experimental and Clinical Infection Research; a joint venture between the Hannover Medical School and the Helmholtz Centre for Infection Research, 30625 Hannover, Germany [5]Department of Clinical Microbiology, Virology & Wallenberg Centre for Molecular Medicine (WCMM), Umeå University, SE-90185 Umeå, Sweden [6]Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Department of Cardiothoracic, Transplantation and Vascular Surgery, REBIRTH - Center for Translational Regenerative Medicine, Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH), German Center for Lung Research (DZL), Hannover Medical School, 30625 Hannover, Germany

	[7]Centre for Pathogen Evolution, Department of Zoology, University of Cambridge, Downing St., Cambridge, CB2 3EJ, U.K [8]Institute of Toxicology, Hannover Medical School, Core Facility Proteomics, 30629 Hannover, Germany [9]Institute of Virology and Immunology (IVI), University of Bern, 3001 Bern, Switzerland [10]Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, University of Bern, 3001 Bern, Switzerland Pharmacological inhibition of the bromodomain and extra-terminal proteins by JQ-1 induces NRF-2-mediated inhibition of SARS-CoV-2 replication in lung epithelial Calu-3 cells and is subject to viral antagonism
10.45-11.00	**Aileen Faist[1]**, Sebastian Schloer[2,3], Angeles Mecate-Zambrano[1], Josua Janowski[1], André Schreiber[1], Yvonne Boergeling[1], Beate Conrad[1], Sriram Kumar[1], Leonie Toebben[1], Klaus Schughart[4], Morris Baumgardt[5], Mirjana Kessler[5,6], Katja Hoenzke[5], Andreas Hocke[5], Marcel Trautmann[7,] Wolfgang Hartmann[7], Ursula Rescher[2], Joachim Kuehn[1], Thorsten Wolff[8], Philip Kuempers[9], Alexandros Rovas[9], Rainer Wiewrodt[10], Peter Barth[7], Stephan Ludwig[1] Linda Brunotte[1] [1]Institute of Virology, University Hospital Münster, Münster, Germany [2]Institute of Medical Biochemistry, University Hospital Münster, Münster, Germany [3]Leibniz Institute for Experimental Virology, Hamburg, Germany [4]Department of Infection Genetics, Helmholtz Centre for Infection Research, Braunschweig, Germany [5]Departments for Infectious Diseases and Respiratory Medicine, Charité University Medicine, Berlin, Germany [6]Department of Gynecology and Obstetrics, Ludwig-Maximilians-University Munich, Munich, Germany [7]Gerhard-Domagk-Institute of Pathology, University Hospital Muenster, Muenster, Germany [8]Robert-Koch-Institute, Berlin, Germany [9]Department of Medicine D, University Hospital Muenster, Muenster, Germany Inhibition of p38 MAPK reduces the SARS-CoV-2 induced inflammatory response and synergistically enhances the antiviral activity of Remdesivir
BRUNCH	
Buses leave for Bologna Airport at 12.00	

Dynamic organelle remodeling: Viral mechanisms to subvert host cell biology

Ileana Cristea

Princeton University, Department of Molecular Biology, Princeton, USA

virtual talk

Spatiotemporal orchestration of herpesvirus morphogenesis

Jens Bosse

Hannover Medical School, RESIST group Quantitative Virology,CSSB Center for Structural Systems Biology, 22607 Hamburg, Germany

Herpesviruses assemble in a complex order of consecutive morphogenesis events. Our research aims to illuminate the spatiotemporal regulation of these morphogenesis events at the single-particle level. To this end, we use three-dimensional live-cell and CLEM as well as biophysical approaches and combine them with computational pipelines to analyze large image datasets. Recently, we have begun integrating neural network-derived predictions to screen for protein-protein interaction involved in herpesvirus morphogenesis and have generated publicly available databases. Here I will illustrate our approach using two recent threads: viral phase separation and bulk egress from infected cells.

Combined Interferon and DNA Damage Signaling Induces Giant PML Cages that Restrict HCMV by Entrapment of Viral Genomes and Capsids

Myriam Scherer[1], Clarissa Read[1,2], Gregor Neusser[3], Christine Kranz[3], Anna K. Kuderna, Regina Müller[4], Florian Full[5], Sonja Wörz[1], Anna Reichel[6], Eva-Maria Schiling[1], Paul Walther[2], Thomas Stamminger[1]

[1] Institute of Virology, Ulm University Medical Center, Ulm, Germany
[2] Central Facility for Electron Microscopy, Ulm University, Ulm, Germany
[3] Institute of Analytical and Bioanalytical Chemistry, Ulm University, Ulm, Germany
[4] Institute of Clinical and Molecular Virology, Friedrich Alexander University Erlangen-Nuremberg, Erlangen, Germany
[5] Institute of Virology, University Medical Center and Faculty of Medicine, Albert-Ludwig-University Freiburg, Freiburg, Germany
[6] Institute for Molecular Medicine, Lisboa, Portugal

PML is the organizer of cellular multi-protein complexes termed PML nuclear bodies (PML-NBs) that confer intrinsic immunity against viruses of different families including human cytomegalovirus (HCMV). Previous studies have shown that PML-NBs associate with HCMV genomes entering the nucleus and restrict viral gene expression by epigenetic silencing. However, PML-NBs are disrupted by viral regulatory proteins such as the immediate-early protein IE1 of HCMV.

Here, we study the antiviral activity of PML-NBs by investigating their association with HCMV input genomes and with newly assembled HCMV capsids in absence of the IE1 protein.

Infection experiments in primary human fibroblast cells revealed that IE1-deleted HCMV induces a drastic rearrangement of PML-NBs to giant, spherical structures referred to as PML cages. These structures are evoked by combined interferon and DNA damage signaling in HCMVΔIE1-infected and also not-infected cells. Visualization of viral input genomes by click-chemistry showed that PML cages are able to entrap HCMV genomes thus leading to impaired viral replication. These PML cages can be disrupted again by expression of IE1 from lentiviral vectors, resulting in increased viral DNA replication and enhanced release of viral particles. Intriguingly, analysis of PML cages at late stages of infection by correlative light and electron microscopy revealed an additional antiviral function, since we detected an unusual clustering of HCMV capsids that correlated with fluorescent signals of PML. 3D EM analysis of whole PML cages showed that a considerable amount of HCMV capsids is entrapped inside several layers of PML fibers. Since predominantly immature viral capsids were encased, we assume that PML cages inhibit virus egress by preventing genome packaging. In summary, our data suggest that giant PML cages are induced by interferon and DNA damage signaling and contribute to HCMV restriction by entrapment of viral genomes as well as newly assembled viral capsids in the nucleus, which is antagonized by the regulatory IE1 protein.

Infection-induced chromatin modifications facilitate translocation of herpes simplex virus capsids to the inner nuclear membrane

Vesa Aho[1*], Sami Salminen[1], Salla Mattola[1], Alka Gupta[1], Felix Flomm[2,3,4,5], Beate Sodeik[4,5], Jens B. Bosse[2,3,4,5] and Maija Vihinen-Ranta[1]

[1] Department of Biological and Environmental Science, Nanoscience Center, University of Jyväskylä, Finland
[2] HPI, Leibniz-Institute for Experimental Virology, Hamburg, Germany
[3] Centre for Structural Systems Biology, Hamburg, Germany
[4] Hannover Medical School, Institute of Virology, Hannover, Germany
[5] Cluster of Excellence RESIST (EXC 2155), Hannover Medical School, Hannover, Germany

Herpes simplex virus capsid assembly and packaging takes place inside the host cell nucleus. Progeny nucleocapsids move in the nucleus by diffusion and must reach the nuclear envelope for egress. Analyzing the capsid movement provides information not only on their transport through nucleoplasm, but also on the properties of nuclear environment during the infection. Here, we combined live-cell imaging and single-particle tracking to characterize capsid motion relative to the host chromatin. Our results indicated that the capsid diffusion was restricted by the surrounding chromatin environment. Capsid motion in the nucleus was subdiffusive and the diffusion coefficients were lower in the chromatin than in regions empty of chromatin. Additionally, the diffusion coefficients in both regions increased during infection. The marginalization of host chromatin towards the nuclear envelope initially presented a restrictive barrier to the capsids, but this barrier became more permeable later in infection, increasing the probability of capsids entering the chromatin. Thus, although chromatin marginalization initially restricted capsid transport to the nuclear envelope, a structural reorganization of the chromatin counteracted it to promote capsid transport later. At no point during the infection were the capsids enriched at the nuclear envelope, suggesting that capsid transport through chromatin is the rate-limiting step for the nuclear egress, instead of nuclear export. This provides motivation for further studies by validating the importance of intranuclear transport to the life cycle of HSV-1.

Spatial, Quantitative and Functional Deconstruction of Protein Interactions inside Cytomegalovirus Particles

Boris Bogdanow, Iris Gruska, Lars Mühlberg, Jonas Protze, Svea Hohensee, Barbara Vetter, Martin Lehmann, Lüder Wiebusch, Fan Liu

Structural interactomics, Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), Berlin, Germany

Virions are infectious units whose infectivity and specificity depend on viral genetic materials, viral proteins as well as co-packaged host proteins. Knowledge about virion composition and structure is therefore critical to understand viral pathogenesis. Herpesviruses assemble particularly large and complex enveloped particles that are difficult to characterize structurally due to their size, fragility and complex packaged proteome with partially amorphous nature. Here, using cross-linking mass spectrometry (XL-MS), we derived a structural interactome of intact extracellular virions of the betaherpesvirus Human Cytomegalovirus (HCMV).

Using the XL-MS method, we revealed a spatially resolved interaction map of 82 host and 36 viral proteins based on ~4,300 crosslinks. Using well-established viral proteins as localization markers, we categorized 36 viral proteins into four spatially resolved layers of the HCMV virion (i.e., capsid, inner tegument, outer tegument and glycoprotein). As an interesting observation, we found that the viral protein pp150 cross-links to proteins from all four layers in a domain-specific manner, indicating this protein is spatially across the entire virion. Furthermore, we found 82 host proteins directly cross-link to viral proteins and they are incorporated into viral particles in a layer-specific manner. To obtain a more comprehensive picture of the proteome landscape, we also obtained the copy numbers of the viral and the co-packaged host proteins using quantitative proteomics. We found that several host proteins are constitutive components that are recruited via specific host-virus interactions. Analyses with viral mutants showed that incorporation of the host phosphatase PP1 and 14-3-3 proteins is mediated by interacting with two short linear motifs (i.e., SILK and RVXF) of the viral protein pp150. PP1 recruitment to HCMV particles influences phosphorylation status inside virions, facilitates the start of viral gene expression and is important for efficient viral replication. Further, at late stage of infection, phosphatase activity regulates the interaction of 14-3-3 proteins with pp150.

Herpesvirus-Host Protein Interaction Contact Site Profiling from Intact Infected Cells by AI-assisted Structural Proteomics

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Protein-protein interactions (PPI’s) facilitate essential processes during viral infections. Accordingly, the global discovery of PPI’s is important to understand the complex nature of host-virus relationships. Despite this importance, no method exists to identify host-virus protein interaction contacts from virus-infected intact cells in a system-wide manner. Here we developed Structural Host Virus Interactome Profiling (SHVIP) that is based on the combination of crosslinking mass-spectrometry (XL-MS) using membrane permeable cross-linker and labeling with bio-orthogonal amino acids during productive viral infection. While XL-MS provides direct PPIs and their interaction contacts from native proteins, enrichment of newly synthesized proteins increases sensitivity of detecting cross-links involving viral proteins. We establish this concept using infection with Herpes-simplex virus type 1 (HSV-1) and find that SHVIP can enhance the proportion of detected crosslinks involving viral proteins by fourfold. We provide an interactome involving ~650 PPIs based on ~7,300 cross-links, which covers ~58 % of HSV-1 proteins including novel and known interactions. Viral membrane proteins, tegument proteins as well as non-structural proteins cluster in different modules in the network dependent on their subcellular location. We complement our data with structural models for the interacting protein pairs based on AlphaFold2 (AF2). Mapping the XL-data onto these predictions indicates reliable structural information of the AF2 models in regions with good confidence metrics (pLDDT > 70) and at least acceptable docking scores (pDockQ > 0.23). We provide a preliminary compendium of ~ 30 structural models involving viral proteins agreeing with XL-data, including UL42-PCNA, UL47-CAPN1, gE-AP1M1, UL45-TPBG, gE-CALR, US2-ANXA2, etc.). Based on these data, we mutated and confirmed binding motifs for 14-3-3 proteins and MAPK8 on the N-terminal domain of the alkaline nuclease UL12. Thus, our data represents the first extensive interactome from intact cells during a viral infection. These data allow independent validation of AF2 predictions and give unprecedented structural insight into host-virus protein interactions at large scale.

The US21 viroporin of Human Cytomegalovirus is a novel regulator of cell adhesion and migration

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Background. During coevolution with its host, the Human Cytomegalovirus (HCMV) has invested a large part of its protein coding potential to ensure the dysregulation of the majority of cellular homeostatic circuits. Among the viral genes involved, the US12 gene family includes a set of 10 contiguous tandemly arranged genes (US12 to US21) and constitutes about 5% of HCMV's genetic content. The identification of putative seven-transmembrane hydrophobic domains in each of the US12 ORFs predicts a common structural framework that associates these proteins with cellular membranes. However, despite the confirmed evolutionary importance of the US12 proteins to HCMV biology, only a few functions in the regulation of virus cell tropism, virion composition, and immunoevasion have been associated with the family to date. Previously, we characterized the function of the US21 protein as a HCMV homolog of cellular TMBIM proteins and an ER-resident virus-encoded calcium-permeable channel able to dysregulate intracellular Ca^{2+} homeostasis and that inhibit apoptosis (Luganini et al., PNAS, 115, E12370, 2018).
Aim of the study. Given the role of Ca^{2+} in controlling cell adhesion and motility, in this study we investigated whether the pUS21-mediated Ca^{2+} release from intracellular stores might influence these important cytobiological responses.
Resuts and conclusions. Initially, we observed that cells infected with TRUS21-HA migrated more rapidly than uninfected cells or TR∆US21-infected cells. We confirmed the results using engineered human cell lines to express pUS21 in an inducible manner, that allows to investigate cell responses without non-specific toxic effects due to a prolonged pUS21 expression, we observed a significant increase in the migration rate of cells expressing pUS21wt, while its mutation in the critical D201 residue that define the pUS21's Ca^{2+} channel leaking function, affected the ability to stimulate cell motility, thus suggesting an involvement of its ability to reduce the Ca^{2+} content in intracellular stores. To test this hypothesis, we performed migration assays in the presence of an inhibitor of calpain 2, a Ca^{2+}-activated cysteine protease, that regulates the disassembly of cellular focal adhesions and promotes cell motility. The addition of the calpain 2 inhibitor abrogated the pUS21-mediated increase of migration in cells expressing this HCMV protein. As a further confirmation of a calpain 2 involvement, its biochemical activity was measured in cell expressing either pUS21wt or its mutated forms in the two critical amino acid residues that define the TMBIM architecture of pUS21, D178 and D201. An increase of calpain 2 activity was observed in extracts from cells expressing pUS21wt or pUS21-D178N, while the expression of pUS21-D201N was unable to stimulate calpain 2 activity, thus confirming an involvement of pUS21 Ca^{2+} channel function in the control of calpain 2 activation. Furthermore, migration assays in the presence of an inhibitor of the store-operated Ca^{2+} entry (SOCE), BTP2, indicate that expression of pUS21 led also to the stimulation of the SOCE mechanism which, in turn, could contribute to calpain activation. Moreover, the functional relationship between pUS21 and calpain 2 was further suggested by the observation that talin 1, a known calpain proteolytic substrate, interact with pUS21 as observed by mass spectrometry, co-immunoprecipitation and immunofluorescence.
Together, these findings highlight a novel role of the pUS21 viroporin of HCMV as a regulator of focal adhesion dynamics, cell adhesion, and migration as a consequence of the dysregulation that pUS21 exerts on the localized Ca^{2+}-dependent activation of calpain 2 and the subsequent talin activity at newly forming protrusions.

Increased vRNA-dependent immune induction conferred by mutations PB1-R386K, PB1-A14V and PB2-N567D in the viral polymerase of avian influenza A viruses

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Increasing evidence suggests that the high pathogenicity of highly pathogenic avian influenza viruses (HPAIV) in humans is partially determined by the production of high amounts of subgenomic viral RNAs that serve as ligands for the pathogen recognition receptors RIG-I, MDA5 as well as PKR and facilitate enhanced induction of type I IFNs and pro-inflammatory cytokines. We previously demonstrated that the transcriptional co-repressor TRIM28 is phosphorylated at the well-characterized inactivating phospho-site serin 473 (S473) during infection with the HPAIV KAN-1 but not by human adapted viruses (Krischuns *et al.*, 2018). S473-P was correlated with increased induction of IFN-b and the pro-inflammatory cytokines IL-6 and IL-8 during viral infection. We could further determine that TRIM28-S473-P is mediated by a signaling cascade constituted of the immune regulating kinases p38 and MSK1. Intriguingly, our data also demonstrated a dependency of S473-P on the activity of PKR, suggesting that virus-derived RNAs enhance the immune response via this non-canonical mechanism. In this study, we identified three avian derived point-mutations in two proteins of the viral polymerase, namely PB1 (A14V and R386K) and PB2 (N567D), which confer enhanced immune induction properties to the human adapted WSN (H1N1) strain. To identify the nature of the immune-stimulatory vRNAs we established a protocol to purify subgenomic vRNAs that harbor intrinsic deletions of various sizes but contain both 3' and 5' promoters directly from lysates of infected cells employing strain specific biotinylated primers targeting all eight genome segments. Captured viral RNAs were further purified using streptavidin-coupled magnetic beads and submitted to Illumina sequencing. We found distinct populations of subgenomic viral RNAs in KAN-1 infected cells 6 hpi, which were highly immune stimulatory upon transfection. These results provide additional compelling evidence for the hypothesis that subgenomic vRNAs act as immune stimulators during infection with HPAIV and that this property is determined by avian amino acid signatures in the viral polymerase.

Keywords: HPAIV, innate immune response, subgenomic viral RNAs, TRIM28

Early recognition of HCMV-pp65 antigen by CD8 T cells

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Human Cytomegalovirus (HCMV) is a betaherpesvirus that latently infects the majority of the human population and can cause severe complications in transplant recipients, newborns, and immunocompromised people. While antivirals targeting HCMV are available, drug resistance frequently develops. CD8 T-cells specific against HCMV antigens (pp65, IE1) presented by HLA class-I molecules are well studied and may provide efficient virus control in immunotherapeutic settings because CD8 T-cells are key components of cellular immunity against HCMV. The ability of TCR Tg CD8 T cells to neutralize HCMV and control its spread, was monitored in a real-time automated fluorescent microscopy. The phosphoprotein pp65 is a late gene encoding for a tegumental protein, but also for a highly immunodominant epitope. We noticed that pp65-specific CD8 T cells were able to control HCMV infection as early as 6 hours post infection (hpi). The early recognition of pp65 antigen presented on HLA-A2 by Tg-pp65 specific CD8 T cells indicated that pp65 is expressed before the late phase of the virus cycle. Monitoring of pp65 gene expression dynamics by reporter fluorescent genes expressed by its promoter revealed that pp65 was detectable as early as 6 hpi, and that a second and much larger bout of expression occurs by 48 hpi. Phosphonoacetic acid blocked this second bout, but not the first one.
Hence, our data suggest that pp65 acts as an early virus gene for immunological purposes.

Impact of glycolytic interference via 2-deoxy-D-glucose on the replication of influenza A virus

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As it is common for viruses, influenza A virus (IAV) elicits considerable modifications of its host cell's metabolism. This includes a substantial increase of the uptake as well as the metabolization of glucose. Even though it's known that interference with glucose metabolism restricts virus replication, data on the exact molecular impact on the viral life cycle are very scarce. Using the inhibitor of glycolysis 2-deoxy-D-glucose (2-DG) we examined how tolerable this treatment is for cells and how exactly the IAV life cycle is impaired by glycolytic interference. We observed the reversibility of 2-DG-induced effects and that cells can cope with high concentrations of the inhibitor by compensating the loss of glycolytic activity by upregulating other metabolic pathways such as cellular respiration. Moreover, mass spectrometry gave information on various metabolic modifications induced by either the virus or 2-DG. In the presence of 2-DG, viral titers were significantly reduced in a dose-dependent manner. The supplementation of direct or indirect glycolysis metabolites led to a partial or almost complete reversion of the inhibitory effect of 2-DG on viral growth and demonstrated that indeed the inhibition of glycolysis and not of N-linked glycosylation was responsible for the observed phenotype. Importantly, we could show via conventional and strand-specific qPCR that the treatment with 2-DG led to a prolonged phase of viral mRNA synthesis while the accumulation of genome copies (vRNA) was strongly reduced. At the same time, minigenome assays showed no signs of a general reduction of replicative capacity of the viral polymerase. Therefore, our data suggest that the significant reduction in IAV replication by glycolytic interference occurs mainly due to an impairment of the dynamic regulation of the viral polymerase which conveys the transition of the enzyme's function from transcription to replication.

Development and implementation of a semi-automated SARS-CoV-2 molecular detection workflow for meeting the demand of daily testing requirements

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To meet daily molecular testing needs for SARS-CoV-2, we used three Alinity_m (Abbott molecular, USA), two cobas 6800 (Roche, Switzerland), and one StarLET (Seegene, South Korea) system. Although a throughput of 5.000 samples per day was achieved, more samples were received daily during the fall and winter months of 2021/2022. The overflows were processed in a manual workflow consisting of: isolation of nucleic acids (NA) using two Maelstrom 9600 (TANBead inc., Taiwan) instruments, manual pipetting of LightMix SARS-CoV-2 E, RdRp gene (TibMolbiol, Germany) and internal EAV control mastermixes separately into 384-well PCR plates, manual addition of NA and running RT-rtPCR in up to three QuantStudio7Pro (ThermoScientific, USA) thermal cyclers. In September 2021, the Janus G3 (PerkinElmer, USA) pipetting robot and LightMix E+N UBC (TibMolbiol, Germany) were introduced into the workflow in conjunction with the same internal EAV control. Dedicated Excel spreadsheets enabled barcoded sample scanning, automatic layout, creation of required templates and linking of results to respective samples, and formation of protocol number strings recognised by LIS for laboratory report generation. After testing 229 samples, the LightMix E+N UBC kit showed 99.6% (95%CI: 97.6% - 99.9%) agreement with previously used kits. Based on 384 comparative samples, the Janus G3 pipetting robot showed better pipetting consistency than humans with range of Ct values for EAV of 3.0 (Janus G3) versus 5.0 (human). In addition, no discrepancies were observed for positive and negative samples. This workflow allowed processing of 9.000 samples per day, with almost no increase in turnaround time (95% - January 2021 vs. 91% - January 2022 of samples processed in <8h), but significantly increased throughput (53.893 – January 2021 vs. 150.592 – January 2022 samples processed) and reduced tedious and error- prone tasks such as pipetting, writing, and typing, allowing laboratory personnel to focus more on analysing results and quality control.

Epidemiological monitoring of SARS-CoV-2 variants circulating in the metropolitan area of Bologna, April-September 2022

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the current worldwide COVID-19 pandemic. The widespread circulation of SARS-CoV-2 has allowed the appearance of emerging viral variants. This study focused on the evaluation of the different lineages circulation in the metropolitan area of Bologna through next generation sequencing (NGS) analysis, from April to September 2022. The study was conducted at Regional Reference Center for Microbiological Emergences, Microbiology Unit, Azienda Ospedaliero-Univeristaria di Bologna (IRCCS). SARS-CoV-2 variants surveillance was performed on 1778 nasopharyngeal swabs (NPS) positive for SARS-CoV-2. Early detection and prevalence of SARS-CoV-2 variants are performed with a diagnostic screening PCR-based assay (Allplex SARS-CoV-2 Variants I & II Assay, *Seegene*). Then Next Generation Sequencing (NGS) is being used as the main tool for epidemiological monitoring of SARS-CoV-2 variants. From April to September 2022, 905 of 1778 NPS are sequenced by NGS technology (Illumina COVIDSEQ Assay, Illumina). Lineage assignment is assessed with both Pangolin and Nextclade.
In this selected period, Omicron variant has been detected in 100% of samples. Therefore, this study focused on Omicron lineage characterisation. In April 2022, BA.1 (11%) and BA.2-like sublineages (85%) were the most common circulating variants of SARS-CoV-2. In May 2022, analysis showed an outburst of BA.4-like variants (29%) which rapidly replaced BA.2-like lineages throughout the following month. BA.4-like sublineages trend was displaced by BA.5-like variants onset (first appearance: May 2022 – 4%) which remained the most represented Omicron sublineage until September 2022 (80 – 95%).
This study provides insights into the rapid change in the epidemiological landscape of SARS-CoV-2 variants in the metropolitan area of Bologna, reinforcing the need of continuous surveillance of viral variants.

HIV latency: the role of G-quadruplex structures in proviral transcription in latently infected cells

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The Human Immunodeficiency virus (HIV) is the etiological agent of AIDS. Although antiretroviral therapy reduces the viral load under detectable level, treatment interruption causes viral rebound because of virus persistence in latently infected cells. How HIV-1 establishes latency is not well understood yet; however, it is well known that the LTR promoter controls the expression of all viral genes. Given the reported presence of G-quadruplex (G4)-folding sequences in the LTR promoter of the HIV-1 provirus and their involvement in transcriptional activation *in vitro*, we here investigated the role of LTR G4s in HIV-1 latency and reactivation. We employed J-Lat Tat-GFP and J-Lat full length cell lines, a valuable cell model to study of HIV-1 latency. The GFP gene downstream the LTR promoter and the use of latency reversing agents (LRAs) allowed us to isolate the transcriptionally silenced proviruses from those transcriptionally active by fluorescence-activated cell sorting (FACS). We next analysed the two cell populations separately and compared LZ`TR G4 folding and G4-ineractors in the two transcriptional states. Through ChIP-qPCR analysis, we demonstrated for the first time that the integrated LTR in the cell chromatin context folds into G4 when in a latent state, whereas it is unfolded when transcriptionally active. We also showed that nucleolin, previously reported to stabilize LTR G4s and inhibit transcription, is indeed bound to LTR only in the latent state. In contrast, the specificity protein 1 (SP1) transcription factor was found bound mainly to the transcriptionally active LTR, devoid of G4s. Taken together, these results uncover a novel mechanism by which G4s, stabilized by nucleolin, inhibit HIV-1 provirus transcription. The involvement of G4s in HIV-1 latency provides a novel potential therapeutic target against HIV infection.

PROVIRAL EFFECTS OF ISG15 AND ANTIVIRAL POTENTIAL OF NRF2 ACTIVATORS AGAINST HUMAN CORONAVIRUS INFECTION IN IPSC-DERIVED VASCULAR ENDOTHELIAL CELLS AND CALU-3 CELLS

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In contrast to the highly pathogenic coronavirus strains, HCoV-229E commonly circulates in the human population and is responsible for approximately 5–10% of all upper and lower respiratory tract infections. HCoV-229E evokes general upper respiratory illness in healthy individuals, but is still capable of causing more severe disease in immunocompromised and older individuals. In order to explore the role of ISG15 in the context with coronavirus infection and the emerging importance of endothelial involvement, we established hiPSC-derived vascular endothelial cells (ECs) as infection model. Contrary to murine ISG15, human ISG15 is known to have proviral effects. For instance, we have previously shown that *ISG15*$^{-/-}$ cells are less susceptible to influenza A virus infection than wild-type cells. We now show that infectivity of hCoV-229E is reduced in *ISG15*$^{-/-}$ ECs, thus adding it to the list of viruses upon which ISG15 exerts a proviral effect. We performed phospho-proteomics of our hCoV-229E infected ECs and found that NRF2 activators modified the expression of stress and inflammation related phospho-proteins. We then assessed the role of the cytoprotective NRF2 signaling pathway in hiPSC-derived WT and *NRF2*$^{-/-}$ ECs. Viral replication was markedly increased in *NRF2*$^{-/-}$ cells, suggesting an antiviral role of this pathway. In order to meet the clinical demand for host-directed antiviral compounds, we tested the antiviral effect of the NRF2 activators bardoxolone-methyl, sulforaphane, and 4-octyl itaconate on HCoV-229E infection. These treatments reduced replication of HCoV-229E, but, strikingly, this antiviral effect was also seen in *NRF2*$^{-/-}$ cells. Additionally, NRF2 activators also reduced replication of SARS-CoV-2 infection in Calu-3 cells. Taken together these results confirm the proviral role of human ISG15, reveal an antiviral role of NRF2 signaling in human coronavirus infection and suggest that the antiviral effects of bona fide NRF2 agonists may be mediated by as yet unidentified NRF2-independent targets. In addition, the results suggest NRF2 activators as adjunct (host-directed) treatment options for infection by coronaviruses.

Chromatin Programming in Herpesvirus Latency

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Herpesviruses package non-chromatinized DNA molecules that are completely devoid of DNA methylation. Starting from a virtually naked and epigenetically naive viral DNA template, latency establishment thus requires *de novo* acquisition and (re-)programming of suitable chromatin states during each round of infection. At the same time, incoming viral genomes must escape host defense pathways that attempt to impose chromatin states leading to irreversible transcriptional silencing and genome elimination.

The regulatory factors and epigenetic pathways governing successful establishment of latent chromatin are only partially understood. We study these processes using infection models of Kaposi sarcoma-associated herpesvirus (KSHV), a virus which very efficiently establishes latency in a wide variety of cell types. To better understand the role of viral and host factors during latency establishment, we use genomically manipulated models in combination with epigenomic and transcriptomic interrogation techniques, as well as live-cell imaging methods to investigate spatiotemporal dynamics of single viral episomes. Our results show that KSHV genomes exploit host pathways normally regulating CpG islands to acquire facultative heterochromatin states which are essential for long-term maintenance of viral DNA. The implications of these findings for our understanding of the role of chromatin programming in DNA virus infections beyond KSHV will be discussed.

Unidirectional recruitment is essential for viral latency

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Kaposi's sarcoma associated herpesvirus (KSHV, HHV-8) is associated with several human malignancies. During latency the viral genomes reside in the nucleus of infected cells as large non-integrated plasmids, known as viral episomes. All KSHV infected cells express LANA, and LANA is essential for viral latency. LANA binding to the viral episomes is critical both for replication of the viral genomes during latency, and for tethering the viral episomes to the cell chromosomes during cell division. Directional recruitment of protein complexes are critical for proper function of many nuclear processes. To test for recruitment directionality between LANA and cellular proteins we directed LANA via catalytically inactive Cas9 (dCas9) to a repeat sequence to obtain easily detectable dots. Then, recruitment of nuclear proteins to these dots can be evaluated. We found that LANA recruited its known interactors ORC2 and SIN3A. Interestingly, LANA was unable to recruit MeCP2, but MeCP2 recruited LANA. Similarly, histone deacetylase 1 (HDAC1) that interact with the transcriptional-repression domain (TRD) of MeCP2, same as LANA, was unable to recruit MeCP2, but MeCP2 was able to recruit HDAC1. In contrast, HP1a that interacts with MeCP2 through a different domain, was able to recruit MeCP2. We propose that available interacting domains in DNA bound/dimerized form of MeCP2, forces this recruitment directionality. We support our model with an elegant experiment, where we provided short DNA oligo with MeCP2 binding site, and under these conditions LANA was able to recruit MeCP2. We found that cells derived from Rett syndrome and express a mutant MeCP2 (T158M), impaired in DNA binding, cannot support KSHV genome maintenance.
Therefore, this unidirectional recruitment of LANA by MeCP2 identified MeCP2 as a critical factor for viral maintenance.

Role of long non-coding RNAs (lncRNAs) in HIV latency

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Therapeutic reversal of HIV latency and elimination of the latent reservoir is the final frontier towards a complete cure of HIV infection. However, current therapeutic strategies fail in settings, and the inability to design effective therapeutic options that control HIV gene expression reflects gaps in our understanding of how HIV transcription and viral latency are regulated. While the mechanism by which host proteins govern HIV gene expression and viral latency have been extensively investigated and are relatively well understood, there remains a significant gap of knowledge regarding the emerging role of the non-coding transcriptome, specicifically long non-coding RNAs (lncRNA) in understanding the steps that establish viral latency.

We present preliminary results that suggest that the lncRNA Cytor is a novel activator of HIV gene expression that is recruited to the HIV promoter and activates HIV gene expression. Cytor also suppresses viral latency establishment and facilitates latency reversal. These functions stem from the direct association of Cytor with the cellular transcription elongation machinery that mediates RNA polymerase pause-release and transcription elongation and modification of the chromatin landscape around the viral promoter. Indirectly, Cytor regulatins down-stream gene targets to promote T cell activation and actine remodelling.

Overall, our study provides noew insights on the regulation of HIV transcription and the role of lncRNAs in the control of viral latency, as we obtain a comprehensive understanding of the role of Cytor in HIV gene expression and latency establishment.

Distinct gene programs underpinning ‘disease tolerance’ and ‘resistance’ in influenza virus infection

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When challenged with an invading pathogen, the host defense response is engaged to eliminate the pathogen (resistance) and to maintain health in the presence of the pathogen (disease tolerance). However, the identification of distinct molecular programs underpinning disease tolerance and resistance remained obscure. Here we exploited interindividual variation in the host to identify central gene programs that act during the infection defense response. We developed and validated the model using transcriptomics and phenotypic data during *in vivo* influenza A virus (IAV) infection of 33 genetically diverse mouse strains that differ in their capacity to resist and tolerate disease, and by integrating high-throughput data of multiple isolated human cell types. The analysis demonstrated one gene program that is associated with the strategy of disease tolerance and another gene program that is associated with the resistance strategy, both at the functional and the phenotypic levels. In accordance, we refer to these programs as 'disease tolerance' and 'resistance' programs, respectively. Systematic analysis of human data across various healthy and inflammatory conditions indicated that the two programs are shared across multiple cell types – including non-immune, innate and adaptive immune cells. Relying on this finding, we developed a generic quantitative metric for the molecular levels of the disease tolerance and resistance programs. This scoring scheme allowed us to identify distinct markers for the activation levels of each program, and subsequently to validate Arhgdia as a regulator of the disease-tolerance program during the host response to IAV infection. Importantly, the baseline level of disease tolerance in peritoneal macrophages is correlated with pathological responses to stress (both injury and infection), suggesting the basal/early disease-tolerance status of macrophages as a druggable driver of disease states. Overall, our approach provides a paradigm for the systematic study of disease-tolerance and resistance states and how they malfunction in complex diseases.

Characterizing the pathogenicity determinants of a clinical influenza B virus isolate from the 2017/18 influenza season

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Seasonal influenza viruses of types A and B cause epidemic outbreaks of severe respiratory disease in humans accounting for up to 5 million infections and 290-650.000 annual deaths worldwide. In the 2017/18 influenza season, influenza B viruses (IBV) of the Yamagata lineage became predominant and caused high numbers of hospitalized patients, which significantly contributed to the high global disease burden and economic costs. While this IBV dominance was partially attributed to a vaccine mismatch, our study demonstrates that additional virus-intrinsic features contribute to the exceptional pathogenicity of these viruses. Direct comparison of the 2018 isolate IBV/Münster/2018/337 with an IBV isolate (Yamagata lineage) from 2016 revealed 31 strain-specific mutations in almost all viral proteins, excluding NP and M1. Results from our experimental analysis provided evidence for increased viral replication at the subpermissive temperature of 37°C in different cell lines and native human lung tissue at 37°C, which indicates improved adaptation to the human lower respiratory tract. Despite increased replication, transcriptome analysis of *ex vivo* infected human lung tissue revealed markedly reduced induction IFN-β and antiviral response genes by the 2018 isolate indicating improved mechanisms of immune antagonism compared to the 2016 isolate. Furthermore, distribution of viral NP in type I pneumocytes following *ex vivo* infection of human lung tissue detected by immunohistochemistry suggested that the IBV 2018 isolate has an increased cell tropism beyond that is not restricted to type II pneumocytes. Combined, the results indicate that the high pathogenicity of the 2017/18 IBV strains is caused by enhanced viral replication in the human lower respiratory tract, which is facilitated by decreased temperature sensitivity, improved immune antagonism and an extended cell tropism. Expanded mutational analysis using reverse genetics is underway to further decipher the contribution of the identified gene-specific mutations to the observed viral phenotype.

mRNA translation – a tug of war during viral infection

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To ensure translation of their mRNAs, viruses commonly hijack the host cell's translation machinery to facilitate viral protein production while concomitantly blocking the cell's ability to mount an immune response. I will present our efforts to pertain the molecular principles governing SARS-CoV-2 ability to suppress host protein synthesis. We further illustrate the viral protein, nsp1, is the main translation shutoff factor of SARS-CoV-2 and we decipher its molecular mechanisms. By generating a viral mutant that lacks nsp1 activity we uncover its functional importance during SARS-CoV-2 infection. We illustrate nsp1 has broad activity and it blocks the translation of all translated cellular mRNA, but its significance lies explicitly in blocking the IFN response.

These results uncover that the multipronged approach SARS CoV-2 is using to interfere with cellular translation and reveal the Achilles heel of the interferon response - its reliance on de novo protein synthesis.

ATRX acts as a modulator of chromatin accessibility following interferon signaling

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The chromatin remodeler ATRX is typically associated with heterochromatic regions, such as telomeres and pericentromeric heterochromatin, and has been shown to be important for maintaining silencing at these sites. Furthermore, ATRX is a component of PML-NBs, which exhibit intrinsic immunity against several viruses by epigenetic silencing mechanisms. As a result, ATRX is targeted by viral regulatory proteins in various ways, such as degradation or displacement from PML-NBs. In this study, we have uncovered an unexpected positive role of ATRX in regulating chromatin accessibility and gene transcription specifically upon interferon (IFN) signaling. Consistent with the reported role of ATRX in heterochromatin maintenance, ATAC-seq analysis revealed that ATRX depletion generally elevated chromatin accessibility. Strikingly, the opposite effect was observed following IFN stimulation: in the majority of the regions chromatin accessibility was reduced in ATRX-depleted cells, suggesting that ATRX positively modulates chromatin structure in response to IFN signaling. In accordance with the changes in chromatin accessibility, whole transcriptome sequencing revealed multiple genes that were less efficiently induced in ATRX-depleted cells upon IFN stimulation. Motif enrichment analysis further indicated a correlation between ATRX-mediated chromatin accessibility and gene expression. By ChIP analysis, we discovered an association of ATRX with the promoter region of *OAS1*, a region that exhibited altered chromatin accessibility in ATRX-depleted cells upon IFN signaling. These results suggest that ATRX associates with specific gene regions in response to IFN signaling and thereby modulates their chromatin environment. In summary, our study provides new insight into the diverse functions of ATRX in regards to chromatin compaction. We hypothesize that specifically upon IFN signaling, ATRX promotes chromatin accessibility at regulatory regions, thereby altering the accessibility for transcription factor binding and thus also gene expression. As a consequence, viral antagonization of ATRX has presumably emerged as an important strategy to broadly compromise intrinsic and innate immune responses.

Unravelling the IFN-α Subtype-Specific Transcriptional and Antiviral Responses to Exogenous Treatment in Native Human Lung Tissues

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Human IFN-α subfamily includes thirteen subtypes with distinct antiviral properties. Due to their biological heterogeneity, IFN-α subtypes have attracted increased attention during the SARS-CoV-2 pandemic, as they potentially qualify as antiviral therapeutics that can be locally-applied to the upper or lower respiratory tracts, where they induce a potent antiviral response against respiratory virus infections. While IFN-α2 is already clinically-licensed for systemic treatments of chronic viral hepatitis, the organ-specific biological reponses and antiviral properties of the other IFN-α subtypes in human lungs remain elusive and urgently require further investigation, to support their clinical translation and therapeutic application. In this study, we compared the transcriptional landscapes of Interferon Induced Genes (ISG) and the antiviral activities of IFN-α subtypes against SARS-CoV-2 and influenza A (IAV) and B (IBV) viruses in native human lung tissues and human respiratory epithelial cells, upon exogenous treatment. In order to acquire quantitative and qualitative information on the subtype-specific gene-expression patterns and antiviral properties, we normalized their immune stimulatory activities using an interferon reporter cell line prior to our experiment.

Our results from transcriptomics reveal that the induced ISG-landscapes fall into three distinct clusters. The first cluster represents the *Core ISGs* of canonical antiviral defence program, which are significantly expressed by all subtypes, although their levels markedly differ between the subtypes. The second cluster represents the *Shared ISGs*, which are significantly expressed by some subtypes but not all, and the last cluster comprises of *Unique ISGs*, which are significantly expressed by only one subtype. According to the average expression levels of the core ISGs, subtypes α2, α8, α14, α16 induce the strongest response, while subtypes α1, α4, α5, α21 induced the weakest response. These expression differences significantly correlated with STAT1 phosphorylation levels 5 min post-stimulation, and also with the antiviral activities of these subtypes against SARS-CoV-2 and IAV, but not against IBV, in Calu3 respiratory epithelial cells.

We also identified that the strong subtypes induced the highest number of both shared and unique genes. Analysis of subtype-specific unique genes revealed non-redundant functions in regulating antiviral and adaptive immune responses, signal transduction regulating inflammatory responses, cytokine/chemokine expression. While α2 induced unique genes regulating innate-immune and antiviral responses (KRTAP3-I, EGR1, FOS, NTRK2), α14 induced unique genes involved in calcium or potassium-regulated signalling and in apoptosis (C2CD4A, KCNN3, GXYLT2, UNC5A). Intriguingly, α8 was the only subtype significantly inducing cytokines and chemokines (CCL5/RANTES, CXCL5). In conclusion, our data reveals that IFN-α subtypes induce a general broad-spectrum antiviral response, as well as non-redundant subtype-specific response in native human lung tissue, corroborating the therapeutic prospects of their functional diversity.

Keywords:

Interferon-α Subtypes, Influenza Viruses, SARS-CoV-2, Transcriptomics, Lung Tissue

Systems biology approaches as leads to unravel the cell biology of papillomavirus entry

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Human papillomaviruses are a large family of non-enveloped double-stranded DNA viruses. Certain so-called high risk types the leading cause for a variety of anogenital and oropharyngeal cancers. Their life cycle is unique in that it requires infected cells to undergo squamous epithelial differentiation. Initial infection or entry into host cells is restricted to the basal stem cells of mucosal or skin epidermis. Entry itself is unique in its own way: viruses bind quickly to heparan sulfate proteoglycans followed by series of structural alterations within the capsid mediated by cellular glycans, chaperones and proteases that allow transfer to an elusive secondary receptor for uptake into cells. Uptake occurs by a novel endocytic mechanism. Intracellularly the virus is routed through endosomes to the Golgi, and imported into the nucleus during mitosis by attaching to mitotic chromatin.

Here, our genome-wide and targeted RNAi screens, as well as proteomic and transcriptomic approaches to study cell biological mediators and restriction factors of entry will be discussed.

Genome-Scale Metabolic Atlas for Emerging and Re-emerging RNA viruses

Ujjwal Neogi

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Viruses rely on their host for replication. The central host metabolic fluxes in the virus-infected cells that affect the viral production were related to flux-balance in the central carbon and energy metabolism and changes in the lipid biosynthesis pathways. RNA virus-host dependencies lie within glycolysis, glutaminolysis, and the citric acid cycle. We aim to develop a genome-scale metabolic (GSM) atlas for emerging and re-emerging viruses, including HIV-1, SARS-CoV-2, Crimean-Congo Hemorrhagic Fever Virus (CCHFV), Dengue, Chikungunya, and Ebola viruses. We have completed for SARS-CoV-2 [Ambikan et al. 2022, ***Cell Systems***], HIV-1 [Ambikan et al. 2022, ***Life Sci Alliance***], and CCHFV [Neogi et al. 2022, ***eLife***]. We have used blood from non-infected and infected patients with different disease severity and performed multi-omics analysis. We inhibited the metabolic pathways pharmacologically to identify the impact on viral replication and reproduction. We identify that all the three viruses use the central metabolic pathways, but the metabolic reactions identified by the flux balance analysis identified different virus-specific reactions.

Our studies offer new possibilities for therapeutic modulation of immune responses through metabolic reprogramming and rationale for developing antivirals against emerging and re-emerging viruses. Further use of integrative omics can thus allow a fruitful route to the computational guiding of experimental antiviral drug discovery and drug repurposing and also understanding the pathogenesis.

Identification of novel host-virus interactions using high throughput screens

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As obligate intracellular pathogens, viruses are completely dependent on the host machinery for their replication. Characterising these interactions contributes to our basic understanding of how viruses work as well as the molecular machinery of the cells they infect. Identification of viral host dependency factors can lead to discovery of clinically relevant small molecular inhibitors with antiviral activity, or, in the case of livestock species, drive generation of pathogen resistant transgenic animals. Evolutionary differences in host-virus interactions can also form barriers against zoonotic and epizootic events and viral adaptations that overcome these barriers underpin pandemic threats. We use high throughput approaches including siRNA, genome-wide CRISPR and arrayed ISG screens, to dissect host-virus interactions in human and livestock species, with particular focus on human cytomegalovirus, influenza virus and more recently coronaviruses. Using cross comparative screens in different species we aim to identify host dependency and host restriction factors that play critical roles in virus replication, the host response to viruses and to gain a better understanding of host jumping events and the viral adaptations that enable them.

MHC class II as novel entry receptor of influenza A viruses

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Despite the availability of vaccines and antivirals, influenza viruses continue to pose a major threat to human health and the economy. In addition to seasonal epidemics, influenza viruses can also cause pandemic outbreaks as a result of zoonotic transmission events. Research in my laboratory focuses on the entry of influenza viruses into host cells, as entry plays a key role in zoonotic transmission. We are particularly interested in the discovery and characterisation of novel entry receptors, which has recently led to the identification of MHC class II complexes as receptors for bat influenza viruses.

In my talk, I will discuss our latest findings on MHC class II complexes as receptors for human influenza viruses and possible implications for zoonotic transmission.

Host cell proteomoe dynamics during coronavirus infection

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Host cells show a wide range of changes during viral infection. These integrate cellular response mechanisms and modulation by the virus. We combine molecular and quantitative proteomics methods to obtain multi-layered information of the system-wide changes brought about by infection. I will present recent findings from cells infected with SARS-CoV-2 or MERS-CoV that show the highly dynamic nature of host cell changes observed. We monitor changes in protein levels, signaling, translation and degradation and combine with systems biology approaches to identify and define pathways crucial for viral replication.

STAR cloning – a new method for rapid cloning of complete herpesvirus genomes

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The herpesviruses comprise numerous human and veterinary pathogens. Their large double-stranded DNA genomes can be cloned as bacterial artificial chromosomes (BACs) and genetically engineered in *Escherichia coli*. While the BAC recombineering methods have become very efficient, the initial cloning step has remained laborious and cumbersome. Consequently, only a limited number of herpesvirus species and strains have been BAC-cloned. Here we show that a simple and efficient cloning method based on transformation-associated recombination in yeast can be used to BAC-clone full-length herpesvirus genomes in a single step. Linear viral genomes were recombined in *Saccharomyces cerevisiae* with a yeast centromeric plasmid and BAC hybrid vector and subsequently transferred to *E. coli*. The entire cloning procedure can be accomplished in less than two weeks. We demonstrated the feasibility of this method by cloning two different betaherpersviruses, rat cytomegalovirus and *Mastomys natalensis* cytomegalovirus as well as two strains of Kaposi sarcoma-associated herpesvirus, a human gammaherpesvirus. The sequence-verified BAC-clones of each virus were used to reconstitute infectious virus in cell culture. This single-step transformation-associated recombination (STAR) cloning method should be broadly applicable to herpesviruses and other large DNA viruses and will greatly facilitate the functional genetic analysis and the use of these viruses as vaccines or therapeutic vectors.

Unravelling mechanisms of T cell pathology in severe COVID-19

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During viral infections efficient T cell activation ensures orchestration of an appropriate anti-viral response and formation of immune memory. However, uncontrolled hyperactivation of T cells can be deleterious and cause target organ damage. It has now become clear that severe COVID-19 is linked to both dysfunctional immune response but also unrestrained immunopathology with T cell responses most likely contributing to disease pathology. To interrogate that further we combined single-cell transcriptomics and single-cell proteomics with mechanistic studies to assess pathogenic T cell functions and inducing signals in acute COVID-19 versus other viral infections. We identified highly activated $CD16^+$ T cells with increased cytotoxic functions in severe COVID-19. CD16 expression enabled immune-complex-mediated, T cell receptor-independent degranulation and cytotoxicity not found in other diseases. $CD16^+$ T cells from COVID-19 patients promoted microvascular endothelial cell injury and release of neutrophil and monocyte chemoattractants. $CD16^+$ T cell clones persisted beyond acute disease maintaining their cytotoxic phenotype. Increased generation of C3a in severe COVID-19 induced activated $CD16^+$ cytotoxic T cells. Proportions of activated $CD16^+$ T cells and plasma levels of complement proteins upstream of C3a were associated with fatal outcome of COVID-19, supporting a pathological role of exacerbated cytotoxicity and complement activation in COVID-19.

Immune responses to HBV and SARS-CoV-2

Matteo Iannacone

San Raffaele Scientific Institute & University, Dynamics of Immune Repsonses, Milan, Italy

State of the art in clinical viral metagenomics – advances and today's challenges

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Clinical metagenomics is slowly becoming part of today's clinician's armamentarium to identify infectious diseases. The clinical application of metagenomics landmarks the revolutionary introduction of this pathogen-agnostic approach, enabling the detection of all pathogens including uncultivable, variant, rare, resistant, and previously undiscovered ones. In this presentation, today's advances and challenges in clinical viral metagenomics are discussed.

Enhanced SARS-CoV-2 entry via UPR-dependent AMPK-related kinase NUAK2

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Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) induces remodelling of the endoplasmic reticulum (ER) for the formation of its replication organelles, thereby causing ER stress and triggering an unfolded protein response (UPR). However, the contribution of individual UPR pathways to infection is poorly defined. Here, we found that SARS-CoV-2 infection causes marginal activation of the signalling sensor IRE1α leading to its phosphorylation, clustering in the form of dense ER membrane rearrangements with embedded membrane openings, and XBP1 splicing. In search for factors differentially regulated by IRE1α-XBP1 during SARS-CoV-2 infection, we identified stress-activated kinase NUAK2 as a novel host-dependency factor for SARS-CoV-2 entry. Reducing NUAK2 abundance or kinase activity impaired SARS-CoV-2 particle binding and internalization by decreasing cell surface ACE2 levels, and viral trafficking likely by modulating the actin cytoskeleton.

Our data suggest that SARS-CoV-2 triggered UPR induces NUAK2, promoting virion binding to cells by maintaining ACE2 cell surface levels and regulating virion trafficking.

Application of pathogen metagenomics - before, during, and after COVID-19

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Emerging viral infections represent one of the greatest threats to global health. They are defined as viral diseases that emerge for the first time in a population or were previously present at a local level but spread rapidly to new geographic areas and populations. Over the past ten years, this threat has become increasingly recognizable. Recurrent outbreaks of Ebola virus disease (EVD) and emerging previously unknown pathogens, such as the Middle East Respiratory Syndrome (MERS) coronavirus and the ongoing pandemic of SARS-CoV-2, have demonstrated the enormous threat such viral diseases pose to our modern society. A large number of potentially pathogenic viruses in the animal reservoir, the risk posed by mutations of the pathogen, and our advancing globalization make this a major threat. Before COVID-19, new technologies, e.g., next-generation sequencing have pointed to ways in which we can better prepare for infectious outbreaks. Especially now, in the era of COVID-19, the importance of pandemic preparedness has increased. In addition to already known pathogens that spill over to humans from animal reservoirs or rapidly mutating pathogens such as influenza, the unknown pathogen or "disease X" plays a unique role as a threat.

The presentation will discuss the benefits and challenges of metagenomics for pandemic preparedness and provide examples of applications.

Proteomic discovery of new prenatal biomarkers which predict the severity of congenital CMV infection

Dana G. Wolf

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Congenital cytomegalovirus (cCMV) is the most common intrauterine infection, leading to infant brain damage. Prognostic assessment of CMV-infected fetuses has remained an ongoing challenge in prenatal care, in the absence of established prenatal biomarkers of cCMV infection severity. Improvements in the diagnosis of primary maternal infection and in prenatal diagnosis of fetal infection, along with the development of new experimental approaches for prenatal antiviral treatment, have triggered extensive de-facto screening of pregnant women for CMV infection in European countries and in Israel.

Together, the diagnostic and therapeutic advances underscore the need for early prognostic biomarkers of cCMV severity. I will present our recent studies in which, addressing this knowledge gap, we have discovered new prenatal biomarkers which predict the severity of cCMV-related fetal brain injury. Employing global proteome analysis along with specific immunoassays in mid-gestation amniotic fluids of CMV-infected fetuses, we have identified the immunomodulatory proteins chemerin and galectin-3-binding-protein as highly accurate prognostic biomarkers, distinguishing fetuses with severe vs asymptomatic cCMV. The findings provide insights for further mechanistic studies of inflammatory pathways and treatable targets involved in the progression of CMV-related fetal brain damage.

Multiple exposure to SARS-CoV-2 enhances cross-neutralizing antibodies to antigenically distinct, non-exposed variants

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Since onset of the COVID-19 pandemic, several SARS-CoV-2 Variants of Concern have emerged, including omicron and its sub-variants. Several studies have shown that SARS-CoV-2 BA.1 omicron is an immune escape variant and current vaccines and infection with pre-omicron variants provide limited protection against BA.1. Meanwhile, however, omicron BA.2 and BA.5 became the dominant variant in many countries and replaced BA.1. As both have several mutations especially in the receptor binding and the N terminal domain compared to BA.1, we analyzed whether BA.2 and BA.5 show further immune escape relative to BA.1. We characterized neutralization profiles against the new BA.2 and BA.5 omicron sub-variants in plasma samples from a variety of individuals with exposure to SARS-CoV-2 by infection or vaccination, including samples from previously virus-naïve, BA.1 or BA.2 omicron-infected individuals. We grouped plasma samples according to their history of exposures in single exposure (unvaccinated and a single infection), two close exposures (two doses of vaccination), two distant exposures (infection with a pre-omicron variant and re-infected with an omicron variant), three or more close exposures (three doses of vaccination or delta variant breakthrough infection), and three or more distant exposures (omicron variant breakthrough infection). To illustrate antigenic differences, we performed antigenic cartography and generated antibody landscapes for all examined study cohorts. Unvaccinated individuals after a single exposure to BA.2 had limited cross-neutralizing antibodies to pre-omicron variants and to other omicron sub-variants. Consequently, our antigenic map, which included all Variants of Concern and BA.1, BA.2 and BA.5 omicron sub-variants, showed that all omicron sub-variants are distinct to pre-omicron variants, but that all omicron sub-variants are also antigenically distinct from each other. The antibody landscapes illustrate that cross-neutralizing antibodies against the whole antigenic space, as described in our maps, are generated only after three or more exposures to antigenically close variants but also after two exposures to antigenically distant variants. Here, we describe the antigenic space inhabited by the relevant SARS-CoV-2 variants, the understanding of which will have important implications for further vaccine strain adaptations.

Evaluation of neutralizing antibodies against SARS-CoV-2 infection

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SARS-CoV2 affects primarily the respiratory system causing a severe form of pneumonia disease called COVID-19. Its pandemic spread to more than 200 million confirmed cases has caused 4.15 Million deaths by now. Though several vaccines have been developed and deployed worldwide, numerous people still await vaccination, while in a percentage of vaccinees immune protection is not achieved. Neutralizing antibodies to treat COVID-19 patients may improve their recovery process. In this study we have tested a SARS-CoV2 neutralizing antibody “STE90-C11”, currently in clinical phase 1 testing. This antibody binds to the receptor binding domain of the SARS CoV-2 spike protein and thus competitively inhibits virus binding to the angiotensin-converting enzyme 2 (ACE2) as receptor on target cells. STE90-C11 neutralizes SARS CoV-2 growth *in vitro* and *in vivo* in the humanized ACE2 (hACE2) mice. Further, it impairs the ACE2 binding of spike from the delta variant of concern (VoC).

We show here the neutralizing effect of this antibody with the new dominant delta variant and its in vivo effects in prophylactic and therapeutic settings.

Pharmacological inhibition of the bromodomain and extra-terminal proteins by JQ-1 induces NRF-2-mediated inhibition of SARS-CoV-2 replication in lung epithelial Calu-3 cells and is subject to viral antagonism

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Inhibitors of bromodomain and extra-terminal proteins (iBETs), including JQ-1, have been suggested as potential therapeutics against SARS-CoV-2 infection. However, molecular mechanisms underlying JQ-1-induced antiviral activity and its susceptibility to viral antagonism remain incompletely understood. iBET treatment transiently inhibited infection by SARS-CoV-2 variants and SARS-CoV, but not MERS-CoV. Our functional assays confirmed JQ-1-mediated downregulation of ACE2 expression and multi-omics analysis (epigenomic, transcriptomic and proteomic) uncovered induction of an antiviral NRF-2-mediated cytoprotective response as an additional antiviral component of JQ-1 treatment. Serial passaging of SARS-CoV-2 in the presence of JQ-1 resulted in predominance of ORF6-deficient variants. JQ-1 antiviral activity was transient in human bronchial airway epithelial cells (hBAECs) treated prior to infection and absent when administered therapeutically. We propose that JQ-1 exerts pleiotropic effects that collectively induce a transient antiviral state that is nullified by an established SARS-CoV-2 infection, raising questions about the clinical suitability of iBETs in the context of COVID-19.

Inhibition of p38 MAPK reduces the SARS-CoV-2 induced inflammatory response and synergistically enhances the antiviral activity of Remdesivir

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Progression to severe COVID-19 following SARS-CoV-2 infection is driven by a dysregulated virus-induced early immune response that culminates in an uncontrolled and harmful expression of pro-inflammatory cytokines. Consequently, patients develop acute respiratory syndrome, multiple organ failure, viral sepsis and have a high risk of succumbing to the disease, as we still lack highly effective anti-inflammatory treatments for this condition. The p38 MAPK plays a key role in the inflammatory response and cytokine storm development during highly pathogenic influenza A virus infections. Suggesting a similar mechanism, we investigate p38 as a potential therapeutic target during SARS-CoV-2 infection utilizing the two clinically tested p38 inhibitors PH-797804 and VX-702 *in vitro* and *ex vivo* in human lung tissue and lung epithelial organoids.

We showed that both inhibitors efficiently blocked SARS-CoV-2 induced downstream signaling of p38. Application of a VSV pseudotype system additionally revealed that p38 is activated in a SARS-CoV-2 spike-dependent manner during the early infection phase. Transcriptomics showed that expression levels of pro-inflammatory cytokines associated with the immunopathogenesis of COVID-19 as well as IFNβ and IFNλ1-3 were significantly reduced by the inhibitors, while expression levels of interferon stimulated genes and IFNα were barely affected. In human lung epithelial organoids, SARS-CoV-2 majorly induced an antiviral response which was largely unaffected by p38 inhibition. Strikingly, we discovered a synergistic effect of both p38 inhibitors in combination with the approved antiviral Remdesivir, strongly reducing viral replication and cytokine expression. A similar synergy with Molnupiravir indicated a general potentiating mechanism of p38 inhibitors with antiviral nucleoside analogs.

These results suggest that p38 inhibition provides a feasible strategy to rebalance a virus-induced overshooting immune response or, in combination with Remdesivir or Molnupiravir, to improve the current antiviral treatments by limiting the risk of viral rebounce and evolution.