1st CroViWo

Croatian Virus Workshop

Basic and Translational Virus Research

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Croatian microbiological society

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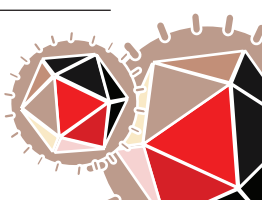

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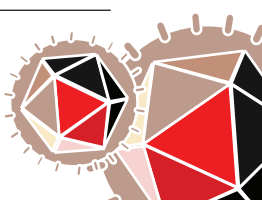

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Foreword

Dear colleagues,

it is our great pleasure to welcome you to the 1st Croatian Virus Workshop!

Viral diseases affect the health of all living beings - humans, animals, plants, fungi and bacteria and thus have an immense socio-economic impact. Although treatment and/or prevention options are available for many viral diseases, there are still many outstanding issues that need to be addressed. It is our wish for this workshop to become a premier event where Croatian and other virologists from the region meet regularly to discuss cutting edge virus research from wide range of topics; molecular virology, host virus interaction and pathogenesis, and clinical efforts in prevention, diagnostics and treatment of viral disease in human, veterinary and agricultural fields. We hope CroViWo will represent a networking and connectivity platform for virologists in Croatia and region with ample opportunities to meet other fellow virologists, learn about their research, methods, and techniques and engage in successful collaborations.

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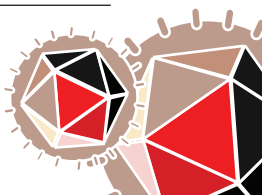

Keynote lecture

Molecular determinants of cytomegalovirus latency in endothelial cells

Franziska Dag (1), Bahram Kasmapour (1), Julia Holzki (1), Adrien Weingärtner (1), Ulfert Rand (1), Lars Dölken(2), Hansjörg Hauser (3), Luka Cicin-Sain (1, 4)

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Cytomegalovirus (CMV) is a major cause of morbidity and mortality in organ transplant recipients. Iatrogenic immune suppression results in poor immune control of latent virus, CMV reactivation from latency, interstitial pneumonia, gastroenteritis and/or end-organ disease. While experiments in the mouse model revealed that lymphocytes play a critical role in the prevention of mouse CMV re-activation, the immune mechanisms playing a role in the immune control of the human CMV remain incompletely understood. Furthermore, the immune mechanisms suppressing viral gene expression in the latently infected cell remain poorly characterized on the molecular level.

To understand the molecular mechanisms driving CMV into latency, we have developed an in vitro model of latency in microvasculature endothelial cells. Namely, cultures of primary human micro-vasculature endothelial cells isolated from the liver or lungs of CMV seropositive patients were PCR-positive in virtually all of the tested patients. Similarly, liver sinusoidal endothelial cells (LSEC) from experimentally infected mice carried mouse CMV (MCMV) and their extensive culture resulted in virus reactivation. In vitro infection of LSECs showed that interferon beta (IFNβ) blocks MCMV gene expression at the immediate-early level in a reversible manner, consistent with the definition of CMV latency. The block occurred due to an upregulation of ND-10 resident proteins, such as DAXX, Sp100 or PML. Importantly, in vivo infection experiments confirmed a key role for IFNβ in the induction of latency at the onset of infection, but also in mice that were latently infected.

In conclusion, our data showed that IFNβ may be sufficient for the induction of CMV latency in a biologically relevant setting, and in cells that naturally harbor latent CMV, both in the experimental model and in clinical settings.

Lectures

Human and primate tumor viruses use PDZ binding as an evolutionary conserved mechanism of targeting cell polarity regulators

Vjekoslav Tomaić (1), Lawrence Banks (1)
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A unique feature of the cancer-causing mucosotropic Human Papillomaviruses is the ability of their E6 proteins to interact with a number of PDZ domain-containing cellular substrates, including the cell polarity regulators hDlg and hScrib. These interactions are essential for the ability of these viruses to induce malignant progression. Rhesus Papillomaviruses are similar to their human counterparts in that they also cause anogenital malignancy in their host, the Rhesus Macaque. However, unlike HPV E6, the RHPV-1 E6 has no PDZ binding motif. This motif specifically confers PDZ-binding activity and directs the interaction of RhPV-1 E7 with the cell polarity regulator Par 3, which it targets for proteasome mediated degradation. These results demonstrate an amazing evolutionary conservation of function between the RhPV-1 and the HPV oncoproteins, where both target proteins for the same cell polarity control network, albeit through different components and pathways.

Infectomics study of human liver non-parenchymal cells in chronic hepatitis C

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INTRODUCTION: Liver non-parenchymal cells (NPC) due to their extraordinary scavenger activity are playing a pivotal role in blood-borne virus clearance. Liver sinusoidal endothelial cells (LSEC) and Kupffer cells (KC) account for the 35% of the hepatic cells and are unique organ-resident cell population with diverse functions, including degradation of bacterial by-products, antigen presentation and induction of tolerance. While these processes are particularly relevant to HCV infection, the role of NPC in chronic hepatitis C is not defined. METHODS: Aim of this study is to apply systems biology approaches to evaluate the role of NPC in HCV infection. Poly(A) RNAs from HCV, MOCK or LPS treated primary LSEC, TPH-1 and hepatoma cell cultures were analyzed by RNA-sequencing (Illumina) to identify differentially expressed genes (DEG) and biological pathways. RESULTS: The system biology approach helped to identify distinct gene expression profiles in HCV infected LSEC, KC and hepatocytes, suggesting their different role in HCV infection. While HCV infection of Huh7.5 cells induced small changes in proinflammatory gene expression, HCV uptake by macrophages induced a dramatic and broad increase in IL1β and NFκB responsive proinflammatory cytokine and chemokines expression, which correlated with increasing severity of liver disease. In contrast, in LSEC the key innate immune response pathways were significantly downregulated, which manifested by the diminished PRR transcripts expression with subsequent tuning down of the expression of the genes encoding for JAK-STAT, NFκB and IRF signaling cascades resulting in the reduced expression of cytokines and reflecting in the deficiencies of innate immune response. However, in LSEC a plethora of immunomodulatory genes were significantly upregulated that might extend the anti-inflammatory effect to surrounding cells and attenuate liver inflammation. Expression of types I interferon and interferon-stimulated genes was unaffected in both LSEC and KC. RNA-seq of Huh7.5 cells identified new pathways that regulate HCV replication in hepatocytes. RNAi knockdown studies on newly identified highly upregulated FUT1 and KLHDC7B genes provide evidence that their gene products regulate

and facilitate HCV replication in hepatocytes. CONCLUSION: This is the first comprehensive gene expression analysis of NPC that provided insight into the broad portrait of genomic changes associated with HCV infection. This approach is likely to have general applications in understanding the host response and viral induced pathology by more comprehensively examining selected biospecimens.

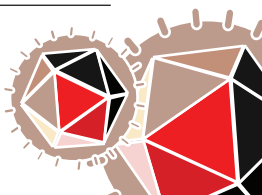
Single, highly abundant transcript of murine cytomegalovirus exhibits coding, non-coding and immune-evasive functions

Vanda Juranić Lisnić (1), Branka Popović (1), Anne L'Hernault (2), Marina Babić Čač (1), Berislav Lisnić (1), Anne Halenius (3), Hartmut Hengel (3), Astrid Krmpotić (1), Lars Dölken (2), Stipan Jonjić (1)

(1) University of Rijeka, Croatia; (2) University of Cambridge, UK; (3) Universitats Klinikum Freiburg, Germany

A large proportion of the herpesviral genes encode immune evasion products that target every aspect of the immune system, ensuring virus persistence and avoidance of detection by the host. Consequently, products of these genes represent potential therapy targets, as well as tools for design of improved antiviral vaccines.

Although numerous immune evasion genes and mechanisms have already been detected and characterized using reverse genetics approaches, recent advances in sequencing technology enabled us to perform comprehensive transcriptomic profiling of murine cytomegalovirus (MCMV) infection of murine embryonic fibroblasts and demonstrate that transcriptional profile and coding potential of herpesviruses have a much greater complexity than previously anticipated. For example, our analysis identified numerous novel spliced and unspliced MCMV transcripts, among which the most highly transcribed viral transcripts are novel transcripts of unknown function. Among these, MAT (Most Abundant Transcript), a single 1.7 kb transcript from the right end of the MCMV's genome, is expressed to the largest extent throughout the infection. It has been shown earlier that MAT encodes a protein (Juranic Lisnic et al (2013) PLOS Pathogens) and contains a binding site for cellular microRNA miR-27 (Marcinowski et al (2012) PLOS Pathogens), making it a first reported viral transcript with coding and non-coding function. We have also recently detected an additional protein encoded by this enigmatic transcript, and here we report that deletion of the MCMV genomic region encoding MAT results in attenuation of the virus in vivo. However, the underlying mechanisms are not completely elucidated due to multiple functions MAT performs in the infected cells. On one hand, MAT deficiency results in failure to deplete cellular levels of miR-27, an important regulator of cell-cycle, while on the other MAT deletion mutants evade the detection by NK cells through activating Ly49 receptors P, L and D2. Furthermore, we also show that absence of MAT results in dramatic changes of host and viral peptides presented in the context of MHC I. In conclusion, not only does MAT provide an excellent example of the complex regulatory potential of herpesviral genes, where single transcripts exerts numerous and diverse functions, it also raises the question of how many new herpesviral genes, especially immune evasion genes, remain to be discovered.



Increased Adenovirus type 5 mediated transgene expression due to RhoB down-regulation

Dragomira Majhen (1), Nikolina Stojanović (1), Dunja Vukić (1), Chantal Pichon (2), Chloé Leduc (2), Maja Osmak (1), Andreja Ambriović-Ristov (1)

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Adenovirus type 5 (Ad5) is a non-enveloped DNA virus frequently used as a gene transfer vector. Efficient Ad5 cell entry depends on the availability of its primary receptor, coxsackie and adenovirus receptor, which is responsible for attachment, and integrins, secondary receptors responsible for adenovirus internalization via clathrin-mediated endocytosis. However, efficacious adenovirus-mediated transgene expression also depends on successful trafficking of Ad5 particles to the nucleus of the target cell. It has been shown that changes occurring in tumor cells during development of resistance to anticancer drugs can be beneficial for adenovirus mediated transgene expression. In this study, using an in vitro model consisting of a parental cell line, human laryngeal carcinoma HEp2 cells, and a cisplatin-resistant clone CK2, we investigated the cause of increased Ad5-mediated transgene expression in CK2 as compared to HEp2 cells. We show that the primary cause of increased Ad5-mediated transgene expression in CK2 cells is not modulation of receptors on the cell surface or change in Ad5wt attachment and/or internalization, but is rather the consequence of decreased RhoB expression. We propose that RhoB plays an important role in Ad5 post-internalization events and more particularly in Ad5 intracellular trafficking. To the best of our knowledge, this is the first study showing changed Ad5 trafficking pattern between cells expressing different amount of RhoB, indicating the role of RhoB in Ad5 intracellular trafficking.

Molecular virology and bacteriology marriage- a case of rape phyllody disease

Martina Šeruga Musić, Silvija Černi, Dijana Škorić

Department of Biology, Division of Microbiology, Faculty of Science, University of Zagreb

Studies in plant pathology often require interdisciplinary research in molecular aspects of unrelated microbes. Oilseed rape (Brassica napus ssp. oleifera (DC.) Metzg.) is important industrial plant and rape phyllody disease, previously ascribed to specific class of bacteria called phytoplasmas, can have devastating effects on its yield. In this case study, plants showing typical rape phyllody symptoms (green and deformed flowers, small and deformed siliques, seed reduction) along with atypical stem necrosis, leaf chlorotic spots and necrotic changes were investigated for the presence of phytoplasmas and viruses. Expectedly, a phytoplasma was identified by PCR-RFLP and sequencing of 16S rRNA gene. Multi-gene sequence characterization of phytoplasma house-keeping tufB, secY, groEL and ribosomal protein genes and the highly variable and specific amp gene was performed. Phylogenetic analyses of all phytoplasma genes confirmed the affiliation of this strain to the 'Candidatus Phytoplasma asteris' (aster yellows, AY) species with the closest relatedness to the 16SrI-B subgroup strains. As opposed to the analyzed house-keeping genes, the amp gene encoding an immunodominant membrane protein, showed a significant variability suggesting possible influence on insect vector specificity and transmissibility. Biological and serological tests revealed the co-infection with Turnip mosaic virus (TuMV). The full genome TuMV sequence obtained for the Croatian isolate CRO184A and its phylogenetic analysis classified it into the world-B phylogenetic lineage, whilst no evidence for inter-lineage recombination was obtained. This way, the first detailed molecular characterization of 'Ca.

P. asteris' associated with the rape phyllody disease was obtained. Also, the first finding of a 'Ca. P. asteris' and TuMV co-infection in oilseed rape, or of any phytoplasma virus simultaneously infecting a brassicaceous host, was presented (Šeruga Musić et al. 2014: J. Appl. Microbiol. 117: 774-785.). In view of the worldwide distribution and pathogenicity of the AY phytoplasma and TuMV as individual plant pathogens, their co-occurrence should be considered in future rape phyllody cases and may have consequences in the disease management, especially if this phytoplasma-virus complex role is demonstrated in the rape phyllody pathophysiology.

Biological control of chestnut blight: effect of host diversity on the prevalence of biocontrol agent – *Cryphonectria hypovirus 1*

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The term "biological control" and its abbreviated synonym "biocontrol" describe the use of pathogen antagonists in suppression of diseases. Biocontrol of a dangerous plant disease, chestnut blight, which is caused by a highly pathogenic fungus *Cryphonectria parasitica* is enabled by naturally-occurring virus *Cryphonectria hypovirus 1* (CHV 1). CHV 1 reduces fungal virulence and reproductive capacities. The fact that this virus attacks only one host species makes it a perfect biocontrol agent. Unfortunately, the spread of this virus is limited by the vegetative (in)compatibility (vc) system of the fungal host. The first comprehensive research of *C. parasitica* in Croatia started ten years ago and revealed considerably higher populations' vc type diversity than in southeastern Europe and slightly lower than in western Europe. The same study also showed high proportion of hypovirulent (CHV 1 – infected) isolates in many tested populations, indicating that CHV 1, responsible for the natural biocontrol of the chestnut blight disease, is widely spread in *C. parasitica* populations. One population from Ozalj, which was included in that study, was observed through the period of ten years. A decade ago, the research showed dominance of a single vc type, EU1, which accounted for more than 60% of all analyzed fungal isolates from that population. Furthermore, little less than half of all analyzed isolates were infected with CHV 1, implying successful natural biocontrol of the disease in this population. However, our recent study in 2014 showed that the EU1 vc type hegemony in Ozalj slowly waned and currently this particular vc type accounts for less than 30% of analyzed isolates. Other vc types' frequencies – most notably EU2 and EU5 increased, while some new, previously unreported vc types emerged. This new genotypes might have migrated from nearby populations or have emerged as a result of sexual reproduction. The most concerning finding of our study was a decrease in percentage of hypovirulent isolates in Ozalj. The obvious reason for that is the fact that the transmission of virus between different vc types is usually difficult or impossible while it occurs with high frequency between the isolates of the same vc type. Therefore, with the increase of fungal vc type diversity, human-mediated biocontrol using well defined CHV 1 isolates (strong and moderate) might be needed to complement naturally-occurring hypovirulence in chestnut protection attempts.

New diagnostic methods allow new insights into epidemiology and diversity of viruses in waters

Maja Ravnikar (1) , Nejc Rački (1), Nataša Mehle (1), Denis Kutnjak (1), Matevž Rupar (1), Jure Papler (1), Petra Kramberger (2), Andrej Steyer (3), Mateja Poljšak Prijatelj (3), Barbara Brajer Humar (4), Marjeta Stražar (4), Ion Gutierrez-Aguirre (1)

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Viruses are the most common biosphere elements in waters. They play an important role in different life processes, including the transfer of genetic elements. To study the presence, survival and between-host transmission of viruses in water, efficient concentration and sensitive diagnostic methods are needed, such as concentration using monolith chromatography and different techniques of amplification and detection of viral nucleic acids (PCR), including digital PCR. Due to the specifics of viral genome, there is a lack of efficient generic diagnostic methods, with the exception of metagenome studies using next generation sequencing (NGS). NGS can be used for viral community studies in the samples and also for discovery of new pathogenic microbes in the environment. Many viruses cannot be easily propagated in host organisms and often high titer of virus is needed to infect a host. Thus, at low viral concentration, problems arise how to detect viral presence and measure their amount, infectivity or success of antiviral procedure. In such conditions, the distinction between assembled virions and viral nucleic acids could pose a problem. Possible solutions of indicated questions will be presented with the examples of plant and human viruses present in the water, using different diagnostic methods.

In situ PCR to detect several porcine parvoviruses

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Porcine parvoviruses (PPVs) are small, non-enveloped icosahedral viruses ubiquitous in different animal species. They are member of family Parvoviridae, genus Parvovirus. Several porcine parvoviruses (PPV) that infect pigs have been identified to date. While PPV1 is well known causative agent of reproductive disorders other porcine parvoviruses were discovered recently. The first of such novel parvoviruses was PPV2 that has been detected in swine serum but not been incriminated in any disease. PPV3 was found in tissue of healthy and sick pigs. PPV4 was detected recently in the lung lavage samples of diseased pigs co-infected with porcine circovirus type 2 (PCV2), collected in 2005. Specific problem was present in research of those novel viruses since they were present in tissue in low viral load. In situ methods that target antigen or nucleic acid such as immunohistochemistry (IHC) and in situ hybridization (ISH) are important and recognized as essential tools for basic research and diagnostics of infection disease. The sensitivity of these methods is fairly compared but limited. ISH is able

to detect 108 genomic copies/g while only103copies/g is necessary using Polymerase chain reaction (PCR). Benefit of in situ PCR has not been extensively explored in veterinary medicine while method is able to detect even one copy of nucleic acid in cell. The aim of this study was to detect novel porcine parvoviruses using in situ PCR. In addition we also applied immunohistochemistry in order to detect whether infected cells are mononuclear inflammatory cells according to nature of parvoviruses. For this study we select tissue samples form 11 pigs that were positive by in vitro PCR for presence of PPV2-4. Results of qRT PCR to detect PPV3 were 102-106 copies. Samples were fixated during 5h, routinely dehydrate and embedded in paraffin block. ISH to detect PPV3 and PPV4 failed. As basis for in situ PCR were previously described methods by Nuovo 2001; Ocadiz-Delgado et al., 2008 and Ocadiz-Delgado et al., 2009 while the cycling conditions and set of primers were adopted from in vitro PCR to detect PPV2-4 by Cadar et al., 2012 and Csagola and et al., 2011. Little modification was made to prevent evaporation during amplification in situ and DIG immune detection was made like in ISH protocol. IHC to detect swine B and T lymphocytes and macrophages were performed using anti-SLAIIDQ, anti-CD3, and anti-lysozyme antibodies. Double staining of cell marker and virus was performed in both ways, in situ PCR than IHC and opposite. We have successfully detected all three viruses in lungs, lymph nodes and liver. Infected cells had compatible morphology to lymphocytes what is according the nature of parvoviruses. Infected cell did not express positive signal for CD3 or lysozyme while were positive for SLAIIDQ cell marker. Real role of infection or did any of investigated viruses compromise lymphocytes in immune function remains unclear.

Molecular epidemiology of human pathogenic "ArboRobo-viruses" in Croatia

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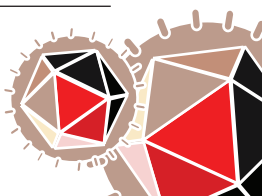
Many zoonotic viruses are transmitted by arthropode vectors (e.g. mosquitoes and ticks) between their reservoir hosts and humans and are therefore called arboviruses. This non-systemic classification comprises approximatley 250 different virus species, 80 of them being pathogenic for humans, for example: Dengue virus or Chikungunya virus. Similarly the term roboviruses describes zoonotic viruses that are being transmitted by rodents. Human pathogenic roboviruses belong to two genera, Hantavirus and Arenavirus.

The newly coined term ArboRoboviruses includes all viruses from both groups that are mostly emerging or re-emerging pathogens. We will focus here on molecular epidemiology of several emerging and re-emerging ArboRoboviruses in Croatia.

Hantaviruses cause hemorrhagic fever with renal syndrome, which was described in Croatia in the



1960's. Since then several epidemics occurred caused by Puumala and Dobrava viruses. Additionally were Tula and Saarema viruses in small rodents detected. The percentage of infected small rodents varies between different hantaviruses and regions and can reach up to 77 %. Molecular epidemiology showed that Puumala virus at higher altitudes of Gorski Kotar represent a phylogenetically distinct clade in comparison to low-land Puumala viruses.

In 2010 three cases of autochthonous Dengue virus infection were discovered at the Pelješac peninsula. Molecular characterization discovered Dengue virus genotype 1 as the causing pathogen, imported by an unrecognized index case, probably from the Indian subcontinent. Retrospective analysis showed this to be the first autochthonous infection of Dengue fever since the 1930's in Europe.

West Nile neuroinvasive disease (WNND), is a serious illness caused by West Nile virus and presents with one of three major clinical syndromes: meningitis, encephalitis or flaccid paralysis. In 2012 the first cases of WNND have been recorded in Croatia. In the following year three times as many cases were hospitalized. In contrast to 2012, where all patients contracted WNND in the eastern parts of Croatia, in 2013 the patients got infected in and around the City of Zagreb. Phylogenetic analysis of parts of the genome showed close relation to strains circulating in neighbouring countries.

A limited molecular analysis of Aedes albopictus and ¬Culex pipiens, from various parts of Croatia, did not show any traces of human pathogenic flavi- or alphaviruses.

Genetic heterogeneity of the tick-borne encephalitis virus in Slovenia

Emina Pustijanac (1), Nataša Knap (2), Luka Fajs (2), Tatjana Avšič-Županc (2)

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Tick-borne encephalitis virus (TBEV) is the most important arboviral agent in central Europe, responsible for thousands of cases of central nervous system infections every year. TBEV is a member of the family Flaviviridae, genus Flavivirus. The genome comprises a single-stranded positive-sense RNA of approximately 11 kb. It is maintained in nature by natural convection between hosts that include small mammals and deer, and ticks - the carriers of the virus. In humans, the dead-end hosts of TBEV, it can cause infection of the central nervous system with serious consequences which has a significant impact on public health. Genetic variability of TBEV has previously been studied predominantly in rodents and ticks, while information about the variability in patients is scarce. The aim of our study was to determine the genetic diversity of TBE virus variants present in TBE endemic regions of Slovenia from tick, rodent and human samples. Viral RNA was isolated directly from samples of ticks and rodents and clinical samples of patients with TBE without indirect cultivation in cell cultures or laboratory animals. We directly sequenced two viral protein genes: NS5 and E. A total of 32 partial NS5 protein gene sequences representing 8 tick, 5 rodent and 19 human samples and 29 partial E protein gene sequences representing 8 tick, 4 rodent and 17 human samples were obtained. The analysis of NS5 and E protein gene sequneces revealed high genetic variability of TBEV and geographical clustering of TBEV in Slovenia. Seven phylogenetic clades were determined both on the NS5 and E protein gene sequence analyses. Our results show that similar TBEV variants are present in all three hosts. Correlation analysis of phylogenetic and geographical clustering showed that ticks and rodents from the same or nearby isolation sites shared a high level of sequence identity, whereas ticks and rodents from different locations showed greater sequence divergence. From records obtained from patients with TBE, we acquired more heterogeneous data. Phylogenetic trees analysis of individual NS5 or

E protein gene sequences revealed that between different genetic lines there was no recombination of the virus except for one important exception which we have seen in a clinical sample of a patient. According to the available information, our study gives the first evidence of possible recombination between different TBEV variants from patients. To the best of our knowledge this is the first study that simultaneously analyzed the genetic relationships of directly sequenced TBEV samples from patients, ticks and rodents in one geographical area.

Deviations from canonical genome organization in different measles virus strains

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Measles virus (MV) is an enveloped RNA virus with single-strand, negative sense, nonsegmented genome. It belongs to genus Morbillivirus within the subfamily Paramyxovirinae, family Paramyxoviridae. The canonical genome organization of MV is characterized by total size of 15 894 nucleotides (nts) and defined length of every region, both coding and non-coding. Same as other members of this subfamily, MV replicates efficiently only when the nucleotide length of its genome is an even multiple of 6 ("rule of 6"). Another characteristic inherent to all viruses belonging to subfamily Paramyxovirinae is precise pseudo-templated nucleotide addition that occurs in two instances during transcription: (a) reiterative copying of short runs of template uridylates in polyadenilation of viral mRNAs; and (b) programmed co-transcriptional insertion of non-templated G residues during transcription of P gene. A pseudo-templated RNA editing mechanism that would be systematically induced during replication has so far not been identified.

Currently, 54 complete genomic MV cDNA sequences can be found in the open public databases, all conforming to the rule of 6. Still, in 10 strains we detected deviations from canonical genome organization due to short, mutually compensating insertions and deletions located within homopolymeric stretches or next to them. These 10 strains can be grouped in 3 clusters, based on their passage histories or epidemiological data. There are no indications that the clusters are somehow evolutionary linked, other than the fact that all strains belong to clade D. In 8 strains the total genome length is unchanged, in 3 strains 7 nts were inserted at one position and 1 was deleted at the other, thereby prolonging the genome for 6 nts.

In all 10 identified strains, one of deviation point is located within the same, 28 nts long segment (positions 5051-5078 in genomic cDNA of canonical strains) that is part of the longest untranslated region in MV genome, indicating that 5051-5078 segment could be involved in preservation of viral genomic structure. This untranslated region, located between ORFs for matix protein and fusion protein, counts for 6.4 % of the total MV genome length and its functions are poorly understood.

A mechanism involved in genome length corrections that occurred in 10 non-canonical strains was either (a) random indel-mutation that restored polyhexameric genome length, followed by a stringent selection for virus in which the correction was close to the point of deviation, or (b) non-random indel-mutation, introduced after the viral replication complex had "sensed" the deviation from the rule of 6. As we found that the same narrow genomic region was mutated in different MV strains, our analysis favours the hypothesis that viral polymerase detects that aberrations during replication have occurred and acts by inserting a correcting mutation at a precise site in the nascent molecule.

CMV expressing NKG2D ligand RAE-1γ employed as a highly immunogenic CD8 T cell vaccine-vector

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Antigen-specific CD8 T cells provide long-term protection against intracellular pathogens and some tumors. This makes CD8 T cell-based vaccines especially attractive option for vaccine design. Cytomegalovirus (CMV) represents the most suitable candidate for CD8 T cell vaccine-vector since it establishes life-long infection which ensures continuous supply of virus specific effector-memory CD8 T cells. Having these facts in mind, we have constructed highly attenuated murine CMV (MCMV) expressing NKG2D ligand RAE-1γ and foreign CD8 T cell epitope. Such a recombinant vaccine-vector provided outstanding CD8 T cell-dependent protective capacity against respective pathogens and maintained this specific response long-term (Trsan et al., PNAS 2013). Although it is generally accepted that the ligation of NKG2D receptor augments CD8 T cell response by providing co-stimulatory signals to CD8 T cells, we showed that the enhanced CD8 T cell response induced by MCMV vector expressing RAE-1γ existed even in mice lacking NKG2D receptor, pointing to an additional, NKG2D-independent immune function of RAE-1γ. Our results indicated that RAE-1γ expression in the context of MCMV vector induced improved antigen presentation via DCs to CD8 T cells. Moreover, RAE-1γMCMV vector was efficient even in N-ras deficient mice, otherwise defective in generating memory CD8 T cells, suggesting that RAE-1γ can circumvent this immune deficit. Altogether, RAE-1γ expressing MCMV demonstrated a powerful capacity to serve as a vaccine-vector. We believe that similar vaccine vectors can be employed to combat various intracellular pathogens and tumors. Since RAE-1γ represents a homologue of human NKG2D ligand ULBP2, one can expect that the results obtained with RAE-1γMCMV vector can be translated to the HCMV vaccine-vector model.

Early immunoreactions in peripheral blood mononuclear cells induced by Puumala virus

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Hantaviruses cause hemorrhagic fever with renal syndrome (HFRS). Infections with hantaviruses are not lytic, and it is currently unknown why infections in humans cause the disease. It is considered that pathogenesis of HFRS is mainly mediated by immune response. The aim of this study was to explore the components of innate and adaptive immunity important in the early peripheral immune response as well as the possible regulatory effect of miRNA on early immunoreactions during the hantaviral infection. Based on this, biological pathways relevant for the immunopathogenesis of HFRS have also been

defined. Using real-time PCR array technology, the relative expression of immune response genes and miRNA was measured in peripheral blood mononuclear cells of patients infected with Puumala virus (PUUV). The results showed suppression of the early immune response to PUUV i.e. genes coding the synthesis of pattern recognition receptors, chemokines and their receptors, cytokines, transcription factors, as well as some signalling molecules. Functional analysis showed that down regulation of the expression of described set of genes modulates inflammatory response by interfering with various cell-signalling pathways. For the first time, the biological importance of miRNA in the regulation of immune response during HFRS was shown. Changes detected at the immune response level have been associated with disease severity but not with the viral load in the blood. MAPK8, CCR5, IL-10, STAT1, STAT4, miR21 and miR19a could be the potential predictors of HFRS pathogenesis.

Deletion of CMV inhibitor of PVR (CD155) results in enhanced virus susceptibility to innate immune control and dramatic attenuation in vivo

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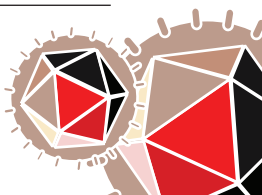
Cytomegaloviruses (CMVs) are well known for their ability to modulate the host immune response by interfering with the expression of cellular ligands recognised by immune cell receptors. One of such ligands is PVR (poliovirus receptor or CD155), a ubiquitously expressed molecule recognised by both activating and inhibitory immune cell receptors: DNAM-1, CD96 and TIGIT. PVR is a non-classical stress induced ligand since it is normally expressed on the majority of somatic cells, but its surface expression can be altered as consequence of viral infections or tumorigenesis. It has been previously shown that HCMV reduces PVR surface expression by retaining it in ER and genes involved have been identified as well (Tomasec et al., 2005). Here we show that MCMV acts similarly by downregulating PVR protein levels from the cell surface. Interestingly, MCMV upregulated transcriptional levels of PVR, suggesting its active regulation of PVR surface expression. We have also characterised the molecular mechanism of this viral regulation that includes PVR retention in endoplasmic reticulum rather than its active removal from the surface of infected cells. In addition, using a panel of MCMV deletion mutants it was possible to attribute this function to a novel MCMV protein within the predicted m20 gene region that we named m20.1. By testing the effect of Δm20.1 viral mutant we showed that retention phenomenon is present in various cell lines, including some immune cells. Moreover, the deletion of m20.1 resulted in a dramatic virus attenuation in vivo, that was possible to attribute to innate immune cells. Altogether, our results indicate the important physiological role of PVR in the immune control of viral infection.



Posters

Dramatic changes in NK cell phenotype and function following perinatal MCMV infection

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Human cytomegalovirus (HCMV) rarely causes an identifiable disease syndrome in immunocompetent hosts. In contrast, HCMV is a frequent cause of acute and chronic disease in individuals with deficits in immunity, including immunologically immature individuals. HCMV is the most common cause of intrauterine viral infection, frequently manifested with life-threatening multi organ diseases. NK cells have been shown to play an important role in host's defense against cytomegaloviruses (CMVs), especially pronounced in early days post infection. In recent years, the adaptive features of NK cells are also being recognized, particularly in response to CMVs. What is the role of NK cells during congenital CMV infection and how is congenital CMV infection influencing NK cell compartment is still mostly unknown. In this study we used mouse CMV (MCMV) infection of newborn mice to test the impact of congenital (perinatal) CMV infection on the maturation and function of NK cells. Our results show that MCMV infection dramatically influences NK cell maturation status and their function.

The antiproliferative effects of chicken anemia virus-derived protein apoptin toward cancer stem cells

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Apoptin, a protein from chicken anemia virus, has the ability to kill human tumor cells. It accumulates in the cytoplasm of normal cells, where it will be subsequently degraded by proteasomal activity. In tumor cells, apoptin enters the nucleus and redirects cell signaling toward apoptosis. It has been found that apoptin senses early stages of malignant transformation, but it is not clear what follows these early changes in cells that would be recognized by the apoptin. In the study presented here, antiproliferative activities of apoptin toward cancer stem cells were compared to its effects toward other tumor cell lines. Transformed primary mammary epithelial cells (HMLE) with silenced gene for E cadherin (HMLE-shEcad) were used as a cancer stem cells (CSC) model. These cells were experimentally induced to pass through epithelial-mesenchymal transition, what was sufficient to acquire stem cells properties. An adenoviral vector that expresses Flag-tagged apoptin gene and a control vector that expresses lacZ gene were used to transfect cells. Localization within the cells, antiproliferative activity, different modes of programmed cell death, and cell cycle disturbances upon Ad-Ap infection were investigated. Although efficiency of transduction was low in the HMLE-shEcad cells, modest growth inhibition was measured. Apoptin localised in the nucleus of HMLE-shEcad cells, but it was not sufficient to induce apoptosis. Apoptin induced cell cycle G2 arrest 24 hours after infection, which delayed cell's growth, but cells overcame this effect and recovered later. Apoptin showed distinct levels of cytotoxicity towards various tumor cell lines, identifying NCI-H1299 (non-small cell lung cancer) as the most sensitive. In these cells apoptin induced apoptosis, and also modulated autophagy. Our study confirmed apoptin as a potent tumor-cell killer, able to modulate different modes of programmed

cell's death, and moreover, to interfere with CSC. Mechanisms of CSC resistance to apoptin should be further analyzed and evaluated in more details.

Sensitivity of mumps virus to the action of IFN-α subtypes

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Human type I interferons (IFNs), predominantly IFN-α and IFN-β, are known to play an important role in early host antiviral response. The IFN-α family consists of even 12 subtypes which despite their high structural homology and signaling through the same receptor have different antiviral, antiproliferative and immunomodulatory activities. Differences in the production of IFN-α subtypes therefore determine the quality of an antiviral response. The aim of this study was to examine the pattern of IFN-α subtypes induced in mumps virus (MuV) infection and then to assess the sensitivity of MuV to the action of IFN-α subtypes. Our results show that all IFN-α subtypes are expressed in response to MuV infection and that pattern of their expression is similar regardless of the virus strain used. Our data demonstrate that all tested IFN-α subtypes can suppress MuV replication, however the intensity and pattern of their action are dependent on a virus strain.

NCR1-deficiency affects the CD4 T follicular helper cell formation and B cell maturation necessary for generation of highly protective antiviral antibodies

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NKp46/NCR1 is an activating NK-cell receptor implicated in the control of various viral and bacterial infections as well as in immune response to tumors. In addition, recent findings suggest a role for this receptor in NK cell education and function but also in shaping of adaptive immune response to pathogens.

To explore immunoregulatory role of NCR1 during viral infection, we first assessed the susceptibility of NCR1 KO (NCR1gfp/gfp), heterozygous (NCR1+/gfp) and wild type (WT) mice to mouse cytomegalovirus (MCMV) infection. Analysis of viral titers at early days post infection showed no difference between NCR1gfp/gfp, NCR1+/gfp and WT mice, suggesting that NCR1 is dispensable for the acute control of MCMV infection. However, the virus titer measured in lungs 14 days p.i. revealed impaired control in NCR1gfp/gfp mice. Furthermore, NCR1gfp/gfp mice had lower number of IFNγ+ NK cells and DCs in lungs 3 days p.i. which may explain the lower frequency of IFNγ+ MCMV-specific CD4 T cells observed in lungs 14 days p.i. In order to determine the importance of NCR1 in antibody response to MCMV, we assessed the frequency of PD1+ICOS+ CD4 T follicular helper cells (Tfh cells) and GL7+ germinal center B cells (GC B cells) in mediastinal lymph nodes (MLNs) of NCR1gfp/gfp and WT mice. As expected, NCR1gfp/gfp mice had lower frequency of Tfh and GC B cells in MLN 7 days p.i. The frequency of mature, isotype switched IgM-CD23+ B cells was also lower in NCR1gfp/gfp mice. In addition, the MCMV-specific antibody response and its protective capacity were inferior in NCR1gfp/gfp mice in comparison to the antibody response in WT control mice.

Our data demonstrated for the first time the role of NCR1 receptor in NK cell-mediated CD4 T cell activation and differentiation to Tfh cells, an essential prerequisite for B cell maturation and generation of antiviral antibodies.

Adenoviruses – good or bad fellows?

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Adenoviruses (AdVs) are icosahedral, non-enveloped viruses with double stranded, linear DNA genome, and they infect a wide variety of vertebrate hosts. Most of the AdVs are apathogenic in healthy individuals, but some can cause disease and even death. On the other hand, because of their biological characteristics, AdVs have a growing popularity as gene delivery and vaccination vectors and also as possible anti-cancer therapeutic agents. For that reason, several EU-financed projects are engaged in understanding the AdV biology and applying them in medicine, one of them being ADVance ITN (Adenoviruses as novel clinical treatments, Marie Curie initial training network). Among all AdVs described so far, human AdV-5 is definitely the best characterized one. However, the human population is widely infected by AdVs and the existing specific antibodies significantly limit the medical usage of HAdVs. Therefore, in the framework of the ADVance, we seek animal AdVs that could serve as appropriate alternatives. Since non human primates are the closest relatives to humans, and the AdVs infecting both groups are very similar, there is an increase of interest in the possible use of simian (monkey and ape) AdVs (SAdVs) in medicine. Chimpanzee AdVs are already well known to be used as vaccine vectors in humans, but many recent studies raised the question of possible host switching of ape AdVs to humans, and the safety of such vectors. The ideal vector virus should be evolutionally and characteristically close enough to HAdVs to be molecularly handled in the same ways, but still far enough to prevent the possibility of crossing the species barrier and infecting humans. Since gorilla and chimpanzee AdVs might be too close relatives to HAdVs, we are looking for AdVs in more ancient primate species: orangutans and gibbons (from the ape group), Old World monkeys (OWM), New World monkeys (NWM) and prosimians, about which there is not much information available. There have been many AdVs found in OWMs, but the majority of them have not been sequenced yet. Regarding the NWMs, only one AdV has been sequenced so far, and there were a few more reports about NWM AdVs, but with hardly any details about them. In prosimian hosts, there have not been any AdVs reported so far. Our laboratory is interested in the taxonomy of the Adenoviridae family as well, which helps a lot in recognizing the differences between virus types and deciding which of them may be better candidates to be studied in more details. The comparative genome analysis of different AdVs would tell us a lot about their diversity, evolution, the function of certain genes, and help us to understand their possible use in medicine. The next big challenge which we have is the isolation and effective propagation of these viruses in different cell lines, as growing capacity of the viruses shows us if they could be used as promising delivery vectors at all. (Support: EU FP7-290002 ADVance ITN)

IL-33 is essential for immunosuppresive Treg cell responses in liver during herpesvirus infection

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Interleukin-33 (IL-33), IL-1 family member, serves as a ligand for the ST2 receptor (Schmitz et al, Immunity, 2005). Following pro-inflammatory stimulation, it is expressed in non-hematopoietic cells (mostly epithelial cells at barrier sites) and released on cell necrosis as an endogenous danger signal, alarmin. Although ST2 receptor is expressed by many immune cells, its expression is highest on mast cells and activated T helper 2 (TH2) cells, where it participates in TH2 immune response by stimulating production of IL-4, IL-5, IL-13 and GM-CSF. However, NK and CD8 T cells also respond to IL-33 with increased IFN-γ production, suggesting its role in TH1 immune response, as well (Smithgall et al, Int Immunol, 2008). In addition, IL-33 is necessary for CD8 T cells (CTL) to control lymphocytic choriomeningitis virus infection (Bonilla et al, Science, 2012). Here we showed that ST2-deficient mice have weaker NK cell and CTL responses to murine cytomegalovirus (MCMV) infection than control wild-type mice. These results suggest that IL-33 signals through ST2 receptor to amplify NK cell and effector T cell responses to MCMV. In spite of this, ST2-deficient mice showed normal viral control, indicating compensatory mechanism induced in absence of ST2. Moreover, ST2-deficient mice infected with MCMV showed much stronger liver pathology, as a consequence of lower percentage of infiltrating Treg cells and lower expression of anti-inflammatory IL-10 cytokine, compared to control mice. These results suggest protective and homeostatic role of IL-33 signaling in inflammation induced by MCMV infection.Sensitivity of mumps virus to the action of IFN-α subtypes

LAMP: isothermal method for fast and simple detection of viruses

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Loop-mediated isothermal amplification method (LAMP) is a molecular method which can be used for fast detection and identification of the microorganisms in the sample. The method is simple and since it is run under isothermal conditions, it can be done in simple heater/reader. The results of the amplification can be determined by the observation of the precipitate in the reaction tube, by change in colour or by emission of the fluorescence. In the latter case, also confirmation of the amplified product can be done. The analysis can be performed in different devices that detect amplification in real-time based on fluorescence, e.g. regular qPCR cyclers and as such, the method is suitable as a fast screening test in diagnostic laboratories. LAMP assay is generally not sensitive to inhibitors and can be used for testing of the crude homogenates, without previous DNA extraction process. In general, the LAMP assays are approximately ten times more sensitive than conventional PCR and ten times less sensitive than qPCR. Due to its simplicity, the LAMP method can be used for on-site or point-of-care diagnostics.

We developed and validated LAMP assays for human and plant viruses.

Human cytomegalovirus (CMV), a member of the family Herpesviridae, is causative for a wide range of diseases. In immunocompromised patients such as organ transplant and HIV/AIDS patients, CMV infections can lead to pneumonia, encephalitis, retinitis, hepatitis, gastroenteritis and colitis. We validated the LAMP assay for specific detection of Cytomegalovirus (HCMV) (eazyplex® CMV, Amplex Biosystems, Germany). The assay has broad dynamic range and was shown to be extremely sensitive, since it detects 15 copies of virus/reaction.

Potato virus Y (PVY) and Pepino mosaic virus (PepMV) are two of the most important plant viruses infecting plants from the Solanaceae family. PVY causes great losses in potato, tobacco, tomato and

pepper production and PepMV is emerging tomato disease worldwide.

LAMP assays for detection of PVY and PepMV viruses were developed previously (Nie, 2005; Ling et al., 2013; Hasiow-Jaroszewska and Borodynko, 2013). Assays were adapted for real-time LAMP format and evaluated by testing several virus isolates. In addition, new LAMP assays were developed to achieve better performance criteria. The best performing assays will be validated in accordance with the EPPO recommendations. Results on the LAMP assay performance characteristics will be presented.

Work was financed by the Slovenian research agency (grant nos. L4-5525 and P4-0165) and the European Metrology Research Programme joint research project "INFECT MET", an EMRP project, jointly funded by the EMRP participating countries within EURAMET and the European Union.

Population structure of Potato virus Y revealed through deep sequencing of virus derived small interfering RNAs and RNA isolated from purified viral particles

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RNA virus populations are one of the fastest evolving biological systems known. When viruses replicate, new mutations are introduced in their population at high rate. Due to the lack of proof-reading ability of their polymerases and frequent recombination events, a cloud of diverse sequences (quasispecies) is generated and retained. It was shown that characteristics of such mutational cloud could influence their host range and virulence and has several other biological implications. New sequencing technologies (next generation sequencing - NGS) dramatically enhanced the resolution of viral population studies. Using NGS, variability of viral populations have been investigated in-depth mostly for few important human and animal pathogenic viruses, but little research has been conducted on plant viruses. The focus of our research is on one of the agriculturally most important plant viruses, Potato virus Y (PVY), a single stranded (ss) RNA virus, a type member of genus Potyvirus. Sequences corresponding to ss RNA viruses infecting plants constitute at least three distinct, but interconnected pools: (I) ss RNA molecules packed in viral particles, (II) double stranded (ds) RNA molecules formed during viral replication and (III) virus derived small interfering RNAs (vsiRNAs). The latter are a result of the well conserved plant defence mechanism called RNA interference (RNAi), in which ds viral RNAs are cut in 21-24 long fragments. We hypothesized that sequence diversity between these pools is similar but could slightly differ due to the errors or genetic bottlenecks introduced during a viral cycle and pools' transitions (e.g. small RNA multiplication, viral packaging). To address this problem, Illumina deep sequencing of two different pools of viral sequences was employed: (1) vsiRNAs and (2) ss RNA isolated from purified viral particles. The data was analyzed to search for low frequency variants and recombination events present in each of the two pools. The results show that both pools reflect highly similar mutational landscape. This indicates sequence-independent targeting of RNAi mechanism towards invading PVY sequences in plants. Nevertheless, some differences were observed between the two sequence pools - small RNAs showing higher level of variation. Further, small RNAs

did not allow to search for recombination events, which were commonly detected in viral particles pool. Observed inter-host differences in viral diversity will enable better understanding of small-scale viral evolution processes.

Removal of waterborne viruses using CIM monolith chromatography

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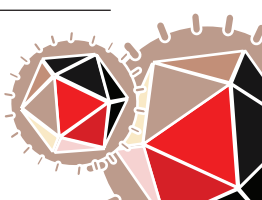
Inefficient waste water treatment, waste water runoffs, natural disasters and similar causes enable the release into the environment of pathogenic microorganisms, such as enteric viruses, that are a major cause of waterborne infections and outbreaks. Infectious viruses are released daily into environmental waters, where, due to their resistance to environmental degradation, they can find their way into potable water sources. Water treatments for virus inactivation or removal are based on physical, chemical, thermal, light-based or membrane technologies. In this study we assessed the potential of CIM monoliths to be used as tool for waterborne virus removal. CIM monolithic supports represent a breakthrough in the concentration and purification of large biomolecules, especially viruses. The particular CIM structure enables convective mass flow what leads to flow independent dynamic binding capacity and separation. CIM monoliths have been successfully applied to the concentration of waterborne viruses where fast flow rates and large binding capacity are essential. Presented results confirm that CIM monoliths can be a valuable tool for waterborne virus removal as well. Removal performance of CIM monoliths was evaluated in effluent from waste water treatment plant on five different enteric viruses that occur in those waters (rotavirus, norovirus genogroup I and II, astrovirus and sapovirus). Effluent waters were chosen as a specially challenging sample to process because of their chemical and biological diversity. They contain a lot of particles and molecules that compete with viruses for binding on a CIM column. During the experiments the CIM based removal method was optimized for longer life time of the column, higher virus binding capacity and lower pressure increase during sample processing.



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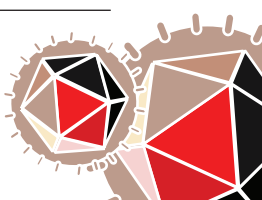

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