THE CROATIAN ACADEMY OF SCIENCES AND ARTS
The Department of Biomedical Sciences in Rijeka
THE UNIVERSITY OF RIJEKA

COVID – 19 MESSAGES I

ADVANCEMENT IN VIROLOGY RESEARCH - AN OPORTUNITY TO IMPROVE INTERNATIONAL IMPACT OF THE UNIVERSITY OF RIJEKA

Rijeka, September 24, 2020
9,30 am
University Campus Rijeka, Faculty of Civil Engineering
Lecture hall G-003, Radmile Matejčić 3, Rijeka

Organizers

THE CROATIAN ACADEMY OF SCIENCES AND ARTS
The Department of Biomedical Sciences in Rijeka

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Daniel Rukavina, president
Snježana Prijić Samaržija, Stipan Jonjić, Bojan Polić

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Registration: online via registration form
for online and onsite participants

Event address for streaming will be sent to all registered participants by e-mail

Free admission but note that the capacity of the lecture hall is restricted. Once all spaces have been filled, no more onsite registrations will be permitted. Participants who want a certificate from the Croatian Medical Chamber need to register.
Refreshments are with no charge.
Parking is free and provided in the building of Student Center Rijeka (Radmile Matejčić 5)

Information

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PROGRAM
OPENING
(9,30 – 10,00)

COVID-19 SYMPOSIA: The Aims and Scope

Snježana Prijić Samaržija, PhD., Professor, Rector, The University of Rijeka, Rijeka, Croatia

INTRODUCTION:Why research of emerging viral diseases needs more serious support?

Daniel Rukavina, M.D., PhD., Professor Emeritus, Head of the Department of Biomedical Sciences in Rijeka, Croatian Academy of Sciences and Arts, Rijeka

10,00 – 12,15 h

I. KEYNOTE LECTURE

Chairman: Stipan Jonjić

Ivan Đikić, M.D., PhD, Professor, Director, Institute for Biochemistry, Goethe University Frankfurt and Max Planck Institute for Biophysics, Frankfurt, Germany
Papain-like protease regulates SARS-CoV-2 viral spread and innate immunity

II. VIROLOGY RESEARCH AT THE UNIVERSITY OF RIJEKA

Chairmen: Pero Lučin and Bojan Polić

Stipan Jonjić, M.D.PhD, Professor, Medical Faculty, the University of Rijeka, Rijeka, Croatia
Scientific achievements and international impact of research in virology at the Faculty of Medicine in Rijeka

Bojan Polić, M.D.PhD, Professor, Medical Faculty, the University of Rijeka, Rijeka, Croatia
Center for translational medical research (TransMedRi) - prerequisite for successful development of immunology and virology in Croatia

Pero Lučin, M.D.PhD, Professor, Medical Faculty, the University of Rijeka, Rijeka, Croatia
Cellular physiology of viral infection

Igor Jurak, PhD, Associate Professor, Department of Biotechnology , the University of Rijeka, Rijeka, Croatia
Department of Biotechnology – from basic to applied research in virology – emergency response to the COVID-19 pandemics

Break for refreshment: 12,15 – 13,00

13,00 – 14,00 h

III. COVID - 19: CLINICS, EPIDEMIOLOGY AND DIAGNOSTICS

Chairmen: Tomislav Rukavina and Igor Jurak

Tomislav Rukavina, M.D., PhD, Professor, Medical Faculty, the University of Rijeka, Rijeka, Croatia
Epidemiological aspects of SARS-COV-2 infection

Vanda Juranić Lisnić, PhD, Assistant Professor, Medical Faculty, The University of Rijeka, Rijeka, Croatia
Diagnostics of SARS-CoV-2 in Rijeka: transforming research laboratory and expertize into clinics

Đurđica Cekinović Grbeša, M.D., PhD, Assistant Professor, Medical Faculty, the University of Rijeka, Rijeka, Croatia
COVID-19 clinical aspects - experience from Infectious diseases Clinic at the Clinical hospital center Rijeka

Coffee break: 14,00 – 14,15

14,15 – 15,15 h

IV. RESEARCH PROJECTS FUNDED BY CROATIAN SCIENCE FOUNDATION

Chairmen: Maja Abram and Vanda Juranić Lisnić

Ilija Brizić, M.D., PhD, Assistant Professor, Center for proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia
No more hiding CoV-2: Establishment of SARS-Cov-2 mAbeom

Astrid Krmpotić, M.D., PhD, Professor, Medical Faculty, The University of Rijeka, Rijeka, Croatia
Highly attenuated β-herpesvirus with potent immunomodulatory capacity as vaccine vector against SARS-CoV-2

Felix Wensveen, M.D., PhD, Associate Professor, Medical Faculty, The University of Rijeka, Rijeka, Croatia
Why we get sick: interactions between the immune and endocrine systems during viral infection

Ana Meštrović, PhD, Associate Professor, Department of Informatics, The University of Rijeka, Rijeka
Multilayer Framework for the Information Spreading Characterization in Social Media during the COVID-19 Crisis

Dalida Rittossa, PhD, Assistant Professor, Faculy of Law, The University of Rijeka, Rijeka
Life in the time of COVID-19 - social implications on the security and well-being of vulnerable groups in the European context

15,15 – 16,00 h

V. ROUND TABLE DISCUSSION: REACHING COMMON GROUNDS

Panelists: Ivan Đikić, Stipan Jonjić, Bojan Polić, Pero Lučin, Igor Jurak, Vladimir Mićović, Snježana Prijić Samaržija and Daniel Rukavina

ABSTRACTS

Introduction: Why research of emerging viral diseases needs more serious support?

Daniel Rukavina
Department of Biomedical Sciences in Rijeka, Croatian Academy of Sciences and Arts, Rijeka, Croatia

In the last six years, from the foundation of the Department of Biomedical Sciences in Rijeka of the Croatian Academy of Sciences and Arts, besides the other activities we have organized 61 scientific conference with the principal goal to get together international scientists of the recognized reputation and croatian scientists, both basic scientists and clinicians, to discuss some of hot topics of the medical science and the clinical practice. The important aim of these meetings is also to support scientists and research groups from Rijeka by promoting interdisciplinary and translational approach and international collaboration, to increase the scientific and medical competencies and to meet challanges of a new University Hospital. In these activities we developed very close and fruitful collaboration with University of Rijeka, Medical Faculty and other University members and Clinical Hospital Center in Rijeka.

Immunology and virology were important topics of these meetings because in Rijeka there is a long tradition and respectable international recognition in these scientific fields. Further, we are delighted with the title of this Conference (COVID 19 MESSAGES 1), which is the first in the series of four, and foccussed to present the internationally recognized achievements of the group of our outstanding scientists and clinicians in the fields of virology and immunology. This is important because at the University of Rijeka works the leading group of croatian virologists which is enormous value not only for the response to actual SARS-COV2 pandemic but particularly for the future activities. The epidemiological dynamics of COVID 19 is fascinating and in a very short time period became pandemic and spread all over the world. This should be looked as an example how fast in the future could spread any new type of virus of uknown characteristics (like SARS-Cov2) and existing scientific potential and knowledge should prepare us to resist and to react immediatelly, not only epidemiologically but by detecting characteristics of viral biology and finding its weak points and working on vaccines. This could be of enormous value for the country and for the future development and international recognition of the University as well. The achievements in the previous period and knowledge accumulated were instrumental in opening new avenues of research of SARS-Cov-2 and Covid 19 in the time when pandemic started. This was additionally recognized by Croatian Science Foundation which decided financially support five research projects of the Rijeka University from 11 accepted for financing altogether. Those five projects will be presented shortly at this conference.

Particularly, I would like to welcome keynote speaker Professor Ivan Đikić, our prominent scientist working in Germany, who is also the member of The International council of Rijeka University. His recent achievements in SARS-Cov2 research and his broader scientific activities could help our efforts to create en environment for establishing in Rijeka an international research center for COVID 19 and new emerging viral diseases.

The Center for translational medical research should be one of the prerequisites in realizing this goal.
Being invoved, more than a half of century, in immunological research I would like to highlight, but very briefly, some aspects of our defence to viral infections based on mechanisms of cell mediated cytotoxicity and particularly the role of perforin (pore forming protein) in resistance to infection. Some two decades ago we discovered characteristics of perforin changes in immunosenescence (Rukavina,D. et al., Blood 92, 2410-2420, 1998) which could be interesting in understanding of some characteristics of COVID 19 development.

Key words: University of Rijeka, immunology, virology, perforin, enlargement of research capacity, smart specialisation

Papain-like protease regulates SARS-CoV-2 viral spread and innate immunity

Ivan Đikić[1,2]
[1]Institute of Biochemistry II, Medical School Goethe University Frankfurt, Frankfurt, Germany
[2]Max Planck Institute for Biophysics, Frankfurt, Germany

The main protease and papain-like protease (PLpro) are essential coronaviral enzymes required for processing of viral polyproteins to generate a functional replicase complex and enable viral spread. PLpro additionally cleaves proteinaceous post-translational modifications from host proteins to evade anti-viral immune responses.
We provided biochemical, structural and functional characterizations of PLpro of SARS-CoV-2 ($CoV2^{PLpro}$) and revealed differences to that of SARS ($SARS^{PLpro}$) in controlling interferon (IFN) and NF-κB pathways. $CoV2^{PLpro}$ and $SARS^{PLpro}$ share 83% sequence identity, yet they exhibit different host substrate preferences - $CoV2^{PLpro}$ preferentially cleaves the ubiquitin-like protein ISG15, whereas $SARS^{PLpro}$ predominantly targets ubiquitin chains. The crystal structure of $CoV2^{PLpro}$ in complex with ISG15 explains high affinity and specificity through distinctive interactions with both ubiquitin-like domains of ISG15. $CoV2^{PLpro}$ is impaired in deubiquitination activities by the lower hydrophobicity of the binding interface with the distal ubiquitin compared to $SARS^{PLpro}$. $CoV2^{PLpro}$ mediates preferential cleavage of ISG15 from interferon responsive factor 3 (IRF3) and reduction of type I interferon responses upon SARS-CoV-2 infection.
Pharmacological inhibition of $CoV2^{PLpro}$ blocks the virus-induced cytopathogenic effect, fosters the anti-viral interferon pathway and reduces viral replication in infected cells. Thus, therapeutic targeting of $CoV2^{PLpro}$ can suppress SARS-CoV-2 infection and promote anti-viral immunity.

Key words: SARS-CoV-2, innate immunity, anti-viral immunity, interferon pathway, papain-like protease

Scientific achievements and international impact of research in virology at the Faculty of Medicine in Rijeka

Stipan Jonjić
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Department of Histology and Embryolog and Center for Proteomics, Rijeka, Croatia

An organized research in virology and viral immunology at the Faculty of Medicine University of Rijeka had effectively started in the late 80's after I have completed the research fellowship at the Federal Research Center for Virus Diseases of Animals in Tübingen, Germany, under the mentorship of Professor Ulrich Koszinowski. Since I was trained as immunologist, I tried to organize my research group initially under the umbrella of the Department of Physiology and Immunology, with particular focus on viral immunology. Later, it became clear that we needed to broaden the scope of our research including viral genetics. I have decided to focus on animal models of viral infections, since we were already highly trained in conducting in vivo experiments and that was why I have picked up a mouse betaherpesvirus – cytomegalovirus (CMV) as a model virus. From the very beginning, my emphasis was not only in understanding of the pathogenesis of acute herpesviral infection, but also viral latency. Starting my own group in Rijeka would be virtually impossible without the unselfish support of the Head of Physiology and Immunology, Professor Daniel Rukavina as well as Professor Ulrich Koszinowski who donated a number of scientific apparatuses and other equipment. In 1996, I became the Head of Department of Histology and Embryology and two members of my group (A. Krmpotić and B. Polić) from the Department of Physiology and Immunology also joined my new department. I should also point out that our start with competitive research would not be possible without an established animal facility allowing us to perform high profile in vivo immunology and viral pathogenesis research. In 2006, with the financial support of the University of Rijeka and the Ministry of Science and Technology we built the Center for Proteomics and the idea was to establish a research department which should base its existence almost entirely on competitive research grants and international scientific collaboration. In addition, the Center made a technological shift towards molecular virology and biochemistry, but also established a high-throughput monoclonal facility generating hundreds of mAbs to viral and cellular antigens, which have been used as a tool in our research, but also in research of our collaborating partners abroad. The key to the success of the Center lies in the fact that we have gradually recruited several top young scientists (T. Lenac Roviš, V. Juranić Lisnić, I. Brizić, B. Lisnić) who became PIs on numerous research projects. The contribution of the team of project managers led by Ani Gerbin was of uttermost importance for applications and administrative managing of many projects. In this summary, I will point out our several major research achievements in virology/ viral immunology published in over 200 of our publications.

I. Immunosurveillance of CMV infection. One of our initial key discoveries was the role of IFN-γ in virus control in vivo (Lučin et al, **J Virol** 1992) as well as the role of TNF-α (Pavić et al, **J Gen Virol** 1993). Subsequent work (Lučin et al, **J GenVirol** 1994) have shown that TNF-α and IFN-γ play a synergistic role in the control of virus infection. Perhaps one of the most remarkable research discoveries of our group at that time was the finding that antiviral antibodies are not required for resolution of primary infection, but for limiting the dissemination of recurrent viral infections (Jonjić et al, **J Exp**

Med 1994). My co-workers Bojan Polić, Pero Lučin and others started to investigate the mechanisms by which CMV interferes with the expression of MHC molecules. A curious finding at that time was also that MHC-I deficient mice have no obvious deficits in the control of CMV infection (Polić et al, **J Gen Virol** 1996), and subsequent studies have managed to explain these studies mechanistically. In the focus of our interest then was the maintenance of herpesvirus latency and dissection of immune response mechanisms involved in this phenomenon (Polić et al, **J Exp Med** 1998). In frame of his PhD thesis Bojan showed that immune control of latency is hierarchically and redundantly organized through different immune cell subsets with T cells playing the major role.

II. Characterization of viral immunoevasion genes. During late 90's we started a series of intensive studies on the role of viral immunoevasins of MHC-I molecules and the CD8 T cell response. We showed that mouse CMV m152 gene product protects the virus from T cell control in vivo (Krmpotić et al, **J Exp Med** 1999). Furthermore, we were first to show that mouse CMV m152 gene product down-modulates a cellular ligand for the activating NK cell receptor NKG2D and play an important role in virus control not only by NK cells with dramatic impact on virus control (Krmpotić et al, **Nat Immunol** 2002). Subsequent studies demonstrated that m152 protein downregulates NKG2D ligand RAE-1γ. Next, we have characterized a viral inhibitor of NKG2D ligand H60 (mouse CMV m155 gene product) and demonstrated the in vivo relevance of its regulation (Hasan et al, **J Virol** 2005). Downregulation of the third NKG2D ligand MULT-1 by viral protein m145 was described by Krmpotić et al, **J Exp Med** 2005. We also showed that CMV Fc receptor m138 downregulates two NKG2D ligands: MULT-1 and H60 (Lenac et al, **J Exp Med** 2006). Furthermore, we have shown that various RAE-1 isoforms are differentially susceptible to CMV immunoevasins, suggesting a continuous evolutional struggle between virus and its host (Arapović et al, **J Virol** 2009). We were also first to demonstrate that CMV encodes genes which interfere with the 'missing-self' recognition by NK cells (Babić et al, **J Exp Med** 2010). More recently, the complex mechanisms involved in avoidance of 'missing-self' has been fully characterized, as well as the viral gene involved. The results of this study solved the long-standing question on how mouse CMV avoids recognition by NK cells. In short, we have characterized new viral immune evasion mechanism which impact the evolution of virus-specific activating MHC I-restricted Ly49 receptors (Železnjak et al, **J Exp Med** 2019). Moreover, in collaboration with the group of Jim Carlyle from University of Toronto we have identified a mouse CMV m12 protein as a functional decoy ligand that engages the NKRP1B inhibitory receptor and inhibits NK cells (Aguilar et al, **Cell** 2017). We have also shown that mouse CMV downregulates the surface expression of PVR (CD155), a cellular ligand for inhibitory receptor TIGIT and activating receptor DNAM-1, and by doing so modulates virus control by inflammatory monocytes and NK cells (Lenac Roviš et al, **J Exp Med** 2016). This finding was key for our subsequent collaborative studies with Professor Ofer Mandelboim from Hebrew University in Jerusalem on the role of antibodies to PVR as a check-point blockers in tumor therapy and setting up the spin-off company Nectin Therapeutics (http://www.nectintx.com).

III. Designing CMV-based vaccine vectors. Based on our findings of viral escape from immune activation, we have designed recombinant CMVs expressing cellular ligand for activating immune receptors inserted in place of their viral inhibitors. Irena Slavuljica in her PhD study showed that such recombinant virus expressing RAE-1γ is

strongly attenuated, but still induces a powerful CD8 T cell response, suggesting that such viruses can be used as vaccine vectors (Slavuljica et al, **J Clin Invest** 2010 and **Front Immunol** 2011). Superior vaccine properties of vector expressing NKG2D ligand RAE-1γ was confirmed in the PhD work of Tihana Tršan (Tršan et al, **Proc Natl Acad Sci U S A** 2013 and **Eur J Immunol** 2017). In collaboration with Professor Martin Messerle, Hannover Medical School, we constructed a similar vector on the backbone of the human CMV (Tomić et al, **PLoS Pathog** 2016). In that respect I would like to point out that the CMV based vaccine vector was recently patent protected (U.S. Pat. No. 10,537,621 approved January 21st, 2020).

IV. Congenital CMV infection. Congenital CMV infections are a leading viral cause of mental retardation and sensorineural hearing loss and represents a significant cause of disease in infants and children. In collaboration with Professor Bill Britt (University of Alabama Birmingham) we have shown that infection of newborn mice recapitulates the hallmarks of congenital human CMV infection (Bradford et al, **PLoS Pathog** 2015; Kosmac et al, **PLoS Pathog** 2013; Koontz et al, **J Exp Med** 2008; Slavuljica et al, **Cell Mol Immunol** 2014; Cekinović et al, **J Virol** 2008). Our recent data show that key drivers of early brain inflammation are NK cells recruited to the brain which secrete IFN-γ that seems to be a major factor of microglia polarization. Although an inflammatory response is the major cause of neurodevelopmental impairment, CD4 and CD8 T cells are essential for virus control and termination of productive infection in the brain (Bantug et al, **J Immunol** 2008; Brizic et al, **Med Microbiol Immunol** 2019). In addition, T cells are retained in the brain as tissue resident memory cells and serve to limit virus reactivation (Brizic et al, **Eur J Immunol** 2018).

V. Translation of CMV research to other research topics and our reaction to Covid-19 pandemics. Several most prominent scientists working in the field of immunology and who finished their PhD under my mentorship continued their research in slightly different domains, still related to CMV biology and other viruses. For instance, Bojan Polić and his team published several very prestigious papers in which they use CMV as a tool to answer some basic questions in the field of immunology (Kavazović et al, **PLoS Biol** 2020; Jelenčić et al, **Nat Immunol** 2018). Bojan and colleagues generated NKG2D knock-out mouse and tested its capacity to control CMV infection (Zafirova et al, **Immunity** 2009). Their achievement in metabolic immunology is remarkable and already recognized in the field (Šestan et al, **Immunity** 2018). They found that virally-induced IFN-γ transiently reduces insulin sensitivity of skeletal muscle and induces compensatory hyperinsulinemia, which promotes antiviral immunity. This leads to persistent glucose intolerance in obese pre-diabetic subjects and cause rapid progression to diabetes.

The development of the cellular biology and cellular physiology research of herpesvirus infection by Pero Lučin and his team at the Department of Physiology and Immunology was associated with early studies of CMV immune evasion mechanisms. These studies were extended to the development of a wide range of experimental settings in the cellular physiology of endosomal trafficking (Mahmutefendić et al, **J Cell Physiol** 2007; Mahmutefendić et al, **Int J Biochem Cell Biol** 2011; Blagojević et al, **J Cell Physiol** 2012; Mahmutefendić et al, **Mol Immunol** 2012), especially endosomal recycling (Mahmutefendić et al, **J Cell Physiol** 2017; Zagorac et al, **J Cell Physiol** 2017), which is also applied in explaining herpesvirus evasion mechanisms (Ilić-Tomaš et al, **J Virol** 2010; Lučin et al, **Cell Mol Immunol** 2014). The lessons learned in the membranous

organelle physiology and immune evasion perturbations of the infected cell opened the area of research related to the understanding of the CMV assembly compartment (Karleuša et al, **Virology** 2018; Lučin et al, **Front Cell Dev Biol** 2018).
Tihana Lenac Roviš at the Center for Proteomics together with Jürgen Haas from Munich, expressed all Varicella Zoster virus proteins and generated monoclonal antibodies against most of them (Lenac Roviš et al, **J Virol** 2013). Vanda Juranić Lisnić was among the first to publish the entire transcriptome of mouse CMV (Juranić Lisnić et al, **PloS Pathog** 2013).
With emerging pandemics of Covid-19, we decided to contribute using different venues. Vanda Juranić Lisnić and Berislav Lisnić with dozen of postdocs and PhD students organized a qPCR testing for SARS-Cov-2 for Clinical Hospital Center Rijeka after the appeal by the National Civil Protection Headquarters. We submitted several grants to the recent call by Croatian Science Foundation on Covid-19 topics and 3 of them have been approved for funding.
In the last 30 years we have worked on more than 35 funded research projects. Projects were funded by different agencies e.g. National Institutes of Health (NIH), European Commission - European Research Council (ERC), Deutsche Forschungsgemeinschaft (DFG). In addition, I am heading the Centre of Excellence "Strengthening the capacity of the Centre of Excellence for Research in Viral Immunology and the Development of New Vaccines" (CERVirVac), in which several groups from University of Zagreb and University of Rijeka are participating and which eventually has received ERDF funding.

Key words: viral pathogenesis, immunosurveillance of viral infection, viral immunoevasion, cytomegalovirus, CD8 T cells, transcriptomics, antigen presentation

Center for translational medical research (TransMedRi) – prerequisite for a successful development of immunology and virology in Croatia

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Department of Histology & Embryology, Rijeka, Croatia

Development of immunology and virology in Rijeka has particular place in development of biomedical science in Croatia. Progress of immunology (since 1965.) at the Faculty of Medicine in Rijeka was one of the preconditions for the first transplantation of kidney (1971.) in the former State. Success of this achievement accompanied with further investments in modern scientific infrastructure, international collaborations, trainings of young scientists abroad and fast implementation of new methods has further boosted experimental research and advancements of immunology. This progress has been followed by a strong development of microbiology, particularly virology, which altogether resulted in significant scientific achievements, high impact publications, recognition at national and international level, and finally with the establishment of the national Center of excellence for viral immunology and vaccines.
More than ten years ago, authorities of the Faculty of Medicine became aware of a necessity to improve collaboration between basic and clinical research groups to facilitate further development. Therefore, the Faculty applied with the infrastructural grant "Upgrading the capacities for research in translational medicine at the Faculty of Medicine University of Rijeka" (TrasMedRi) to EU-FP7-Regpot call in 2009. The project was

granted and successfully implemented (2010 – 2013). It significantly improved our research capacities for translational research in immunology/inflammation, infectious diseases, and cancer. To continue and further develop the activities after completion of this project, the Faculty and University of Rijeka launched an initiative to build up Center for translational medical research - TransMedRi at the University Campus as a research institute which would be important part of the future "Health complex" and an essential link between the institutions involved in biomedical research and education at the University.
TransMedRi has been conceived as a joint research institute of the Faculty of Medicine in Rijeka, Clinical hospital Center Rijeka and the University of Rijeka. TransMedRi is meant to be internationally visible institution based on the translational medical research in the field of immunology/inflammation interdisciplinary intertwined with infectious diseases, oncology, metabolism and neurology. In the focus of research will be mechanisms of immunosrveillance of specific dangerous pathogens (i.e. SARS-CoV2) and development of vaccines, cancer immunotherapy, mechanisms of inflammation standing behind chronic metabolic and neurological diseases and development of different immunotherapeutics. This research will be organized within five departments such as Department for immunity to pathogens and vaccines, Department for cancer immunotherapy, Department for metabolic diseases, Department for neurological diseases and Department for clinical trials phase 1 & 2. In addition, TransMedRi will possess BSL3 animal facility and laboratories, biobank, functional magnetic resonance imaging (fMRI) unit and several other core facilities.
Altogether, the establishment of TransMedRi is an essential step forward for development of a top translational research in immunology and virology in Rijeka and Croatia.

Key words: translational medicine, immunology, virology, BSL3 laboratories and animal facility, clinical trials phase 1 and 2, biobank

Cellular physiology of viral infection

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University of Rijeka Faculty of Medicine, Department of Physiology and Immunology, Rijeka, Croatia

Virus infection extensively reorganizes host-cell functions and establishes new physiological circuits aligned with the needs of viral replication. The extensity of reorganizations is associated with the coding potential of a virus that targets host-cell functions. Members of the Herpesvirus family possess one of the most considerable coding potentials among viruses, especially members of the beta-herpesvirus family. They extensively reorganize almost all cellular functions in order to establish a virus factory, the manufacturing process of assembly, and the shedding of newly formed virions. These include development of new nuclear structures and compartments required for viral DNA replication and virion assembly, reorganization of the membranous organelles of the cell into the new structure known as assembly compartment, adaptation of the cell volume and shedding of a large amount of membranous structures, rearrangements of the cell architecture by cytoskeleton remodeling, adaptation of cell energetics and reorganization of mitochondria, reorganization of cell degradation system including autophagic machinery, cellular signaling, and cell death-associated physiological cir-

cuits. Additionally, the reconfiguration of the host-cell organization is associated with the alteration of many physiological processes, which has a consequence not only on the entire cellular physiology but also to the higher-order physiology. These result in an adaptation of the infected cell to the system processes within an organism, which affects the pathophysiology of infection, such as immune evasion and modulation of the inflammatory response.
All these reorganizations are associated with the rearrangement of the host-cell transcriptome, proteome, and lipidome, which represent the basis for reconfiguration of almost all physiological circuits within the infected cell. For example, membranous organelle system of the cell is shaped by more than three thousand host-cell factors (genes encoding proteins), and beta-herpesviruses alter the expression of almost 1500 of them. Thus, understanding the structure of physiological circuits, organization of effector and regulatory networks, and spatial and temporal interactome within these networks is essential for understanding the physiology and pathophysiology of virus infection. Although, an extensive systems studies (i.e., transcriptomic, and proteomic) conducted in the last decade gained insights into the complexity, we are still far from the full understanding of many cellular physiology processes within the infected cell.
In this lecture, we will present our current understanding of the reorganization of membranous organelle systems in the beta-herpesvirus infected cell into the assembly compartment, a structure as large as the nucleus, which gradually evolves in the course of infection. This structure is required for final stages of the beta-herpesvirus manufacturing, the secondary envelopment and virion egress, and represent the basis for many alterations of cellular and the higher-order physiology.

Keywords: cellular physiology of virus infection, beta-herpesviruses, assembly compartment, membranous system of the cell, host-cell proteome

Department of Biotechnology – from basic to applied research in virology – emergency response to the COVID-19 pandemics

Igor Jurak
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Since the outbreak of atypical pneumonia cases late in 2019 in the Wuhan region SARS-CoV-2 has spread all over the World infecting millions of people. The first confirmed cases in Croatia were identified two months after the initial outbreak, which gradually lead to of implementation of strict epidemiological measures to limit virus spread. To characterize the circulating viruses in Croatia an interdisciplinary group of scientists from three different institutions including Department of Biotechnology, Teaching Institute of Public Health (TIPH)/Medical Faculty in Rijeka and Institute Ruđer Bošković (IRB) sequenced 21 SARS-CoV-2 samples. Our results showed evidences of multifocal introduction of virus already during early times of spring 2020 epidemics in Croatia. Important to mention, all three participating institutions have brought specific expertise to successfully and efficiently complete the goal. While TIPH and IRB were essential for obtaining the samples and sequencing, respectively, scientists of the Department of Biotechnology (BioTech) initiated the project and analyzed the DATA. The Department is well equipped for research in various fields, including molecular biology, biomedicine, and chemistry, and in the vicinity are excellent computational re-

sources (super-computer BURA). Virology research is limited to only the Laboratory for molecular virology, headed by Igor Jurak. Although the main focus of the group is the molecular biology of herpesviruses, namely control of gene expression, miRNAs and RNA modifications; we have successfully applied our experience to respond to the imminent need to contribute in understanding the COVID-19 pandemics. An overview of resources of the Department of Biotechnology and virology research will be presented.

Key words: SARS-CoV-2, sequencing, mutations, Department of Biotechnology

Epidemiological features of SARS-CoV-2 infection

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Department of Social Medicine, Rijeka, Croatia
[2]Epidemiology Teaching Institute of Public Health of Primorsko-goranska County,
Department of Molecular Diagnostics, Rijeka, Croatia

The aim of this presentation is to present the most recent epidemiological features of COVID-19 infection caused by the SARS-CoV-2 virus. The infection was declared a pandemic by the World Health Organization (WHO) on March 11, 2020. The pandemic was rapidly spread worldwide. Figures from July 22, 2020 show that more than 15 million people had been infected with the virus, causing more than 620,000 deaths in most of the countries of the world.

It was discovered that the infection is caused by a Novel Coronavirus (SARS-CoV-2) and that it probably originated in Wuhan, China. The Wuhan health authorities detected few cases of an atypical pneumonia in mid-December 2019. The preliminary conclusion was that the causative agent probably jumped from an animal reservoir to a human during the first week of November 2019, although the specific origin of this new pandemic is not totally understood. Several investigations were focused on identification of animals responsible for these new zoonotic diseases. Although it still remains unclear which animal is the intermediary host, it is well-known that bats are the main reservoirs for these types of virus and that they probably emerged in one of the local wild-animal farms.

A series of research concluded that the transmission of COVID-19 is by droplets or by being in contact with exposed surfaces. A significant proportion of transmissions occur secondary to exposure to an asymptomatic people and the patient can transmit the infection up to 2 weeks after having recovered from symptoms of the disease.

The epidemiological dynamics of COVID-19 has changed dramatically over the course of developing pandemic. At the beginning the most affected continent was Asia, with China being the most affected country. Nowadays, the Americas have converted to the region which is the most affected.

The basic reproduction number (R_0) as a measure that quantifies the epidemic potential of a pathogen for SARS-CoV-2 ranges between 2 to more than 3, numbers that vary depending on a series of parameters. The incubation period of COVID-19 typically ranges from 2 to 14 days (98% of patients), with an average of 5 days, although there have been cases with incubations periods of up to 24 days. Typically, the time from infection onset to development of severe disease is one week.

Key words: animal reservoir; COVID-19; epidemiology; pandemic; reproduction number (R_0); SARS-CoV-2 virus

Diagnostics of SARS-CoV-2 in Rijeka: transforming research laboratory and expertise into clinics

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged late in 2019 and has managed to spread to every corner of the world in only a few months. By summer 2020 it has infected millions of people worldwide, caused thousands of deaths and might cause numerous, as of yet not fully understood, sequelae. One of the reasons for the rapid spread of SARS-CoV-2 lies in the recently discovered fact that even pre-symptomatic and asymptomatic individuals may shed the virus at a very high rate. These latest developments underscore the need for rapid, sensitive, and specific diagnostic tests as well as for skilled virologists, capable of a rapid response, development and implementation of diagnostic tests before ready-made kits and point-of-care systems become available. In this talk, I will highlight advantages and disadvantages of various available diagnostic tests and describe a course of implementation and validation of a diagnostic test in frame of a purely academic research laboratory. I will also share our experience in performing diagnostics for Clinical hospital Rijeka.

Key words: SARS-CoV-2, COVID-19, diagnostics, ELISA; serology, qPCR, RT-qPCR

COVID-19 clinical aspects - experience from Infectious diseases Clinic at the Clinical hospital center Rijeka

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Since the beginning of 2020. novel coronavirus, simply named SARS-CoV-2 has efficiently spread throughout the world, causing pandemic. Infectious diseases Clinic of the Clinical Hospital Center Rijeka welcomed first SARS-CoV-2 positive patient on 25th of February 2020. Until this day, 62 patients have been hospitalized in the Clinic for purpose of monitoring and treating COVID-19 disease. Here we will present the course of the disease, treatment and outcome of patients with emphasis on clinical manifestations variety and importance of close patient monitoring due to possible rapid deterioration in later time points of infection.

Key words: COVID-19, clinical presentation, pneumonia, SARS-CoV-2 virus

No more hiding CoV-2: Establishment of SARS-Cov-2 mAbeom

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In late December of 2019, the coronavirus disease (COVID-19) caused by severe acute respiratory syndrome CoV-2 (SARS-CoV-2) was identified in Wuhan (China). Since then, SARS-CoV-2 has been declared as public health emergency, quickly spread all

over the world and pandemic of SARS-CoV-2 was declared on March 11th 2020 by the World Health Organization (WHO). Basic and applied research of SARS-CoV-2 biology and pathogenesis is of utmost need. Many academic and industry laboratories worldwide have employed their capacities to deal with SARS-CoV-2, resulting in major gain of knowledge in only a few months. However, further progress and understanding of SARS-CoV-2 will heavily depend on development of research tools. Monoclonal antibodies are an essential tool in most life science studies, widely used in both diagnostics and therapy.

Our goal is to develop monoclonal antibodies for all of SARS-CoV-2 proteins. To that aim we will: 1) Express all SARS-CoV-2 proteins (whole proteome, 29 proteins) using eukaryotic and/or prokaryotic expression system; 2) Produce monoclonal antibodies to all SARS-CoV-2 proteins (produce SARS-CoV-2 mAbeom); 3) Validate monoclonal antibodies and implement them into research of SARS-CoV-2. To achieve these goals we have established collaboration with several national and international research groups working on complementary research. Since the research of SARS-CoV-2 is rapidly expanding, existence of these antibodies will enable to meet emerging needs of scientific community all over the world. Importantly, development of such tools has a huge potential to significantly enhance the ability of scientific community to study basic biology of the virus, but also to join global scientific efforts to cope with the pandemic.

Keywords: SARS-CoV-2; monoclonal antibodies; proteins; proteome; COVID-19

Highly attenuated β-herpesvirus with potent immunomodulatory capacity as vaccine vector against SARS-CoV-2

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Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), appeared in China at the end of 2019 and has rapidly increased into global pandemic and a major public health issue. COVID-19 symptoms **can** range **from very mild to severe illness.** An effective anti SARS-CoV-2 treatment has not been identified yet and there is an urgent need to develop an effective vaccine against this virus. Studies on other coronaviruses indicate that viral structural proteins, with many B cell and T cell epitopes, are the most immunogenic. Data published so far on immune responses against SARS-CoV-2 indicate that in addition to antibody response, T cells, especially those residing in the respiratory tract, are important for SARS-CoV-2 immune control providing long-term protection.

Human cytomegalovirus (HCMV) is **β**-herpesvirus that is widely present in the human population, but infection is usually asymptomatic in immunocompetent individuals. CMV infection induces a unique CD8 T cell response in a process known as «memory inflation» - CD8 T cells specific for certain viral epitopes continue to expand during viral latency and eventually stabilize at high frequencies. In addition, CMV-specific CD8 T cells are biased toward mucosal tissue distribution, which is relevant for the control of pathogens at their sites of entry. Our group has been studied for years many immunoevasion mechanisms that CMVs develop to avoid host's immune control. Based on the data obtained on immunology of recombinant CMVs lacking immunoevasion genes, we have constructed recombinant CMV-based vaccine vectors. We

designed recombinant mouse CMV (MCMV) in which RAE-1γ, a ligand of activating immune receptor NKG2D, was inserted into the viral genome in place of its inhibitor (RAE-1γMCMV). This virus is highly attenuated but at the same time is able to generate highly protective antibody as well as T cell response. We have also generated RAE1γMCMV-based vectors expressing foreign epitopes (e.g. immunodominant CD8 T cell epitope of L. monocytogenes) and confirmed that the expression of the NKG2D ligand RAE-1γ by an MCMV vector bearing a foreign CD8 T cell epitope significantly increased the magnitude and effector functions of the CD8 T cell response.
All in all, our data provide strong evidence that CMV expressing cellular ligand for NKG2D receptor represents an excellent vaccine vector against emerging pathogens, such as SACS-CoV-2. Therefore, the major goal of the project is to generate recombinant RAE-1γMCMVs expressing S, M or N structural proteins of SARS-CoV-2 and to investigate their vaccine potential.

Key words: cytomegalovirus, SARS-CoV-2, vaccine vector, CD8 T lymphocytes, NKG2D

Why we get sick: interactions between the immune and endocrine systems during viral infection

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Being sick makes us miserable. Following infection with a pathogen such as SARS-CoV-2, the cause of COVID-19, we lose apetite, get a temperature and feel weak. Whereas we experience these feelings as pathology, they are in fact a carefully orchestrated response. Upon infection, the immune and endocrine system communicate to change systemic metabolism and induce a state that we experience as 'being sick'. The purpose of this system is to impair replication of the invading pathogen and promote the immune response, most notably by CD8 T cells. The underlying molecular mechanism of this process have long remained unknown, but recent advances have made clear how the immune system mediates changes in endocrine function upon infection. In the context of pre-existing metabolic disease, this system derails and may promote development of pathologies such as diabetes mellitus type 2 (DM2). Importantly, patients with metabolic disease fail to induce the immune-mediated anti-viral changes in systemic metabolism, which predisposes them to severe disease outcome following infection with pathogens such as SARS-CoV-2. Indeed, DM2 is one of the biggest risk factors for morbidity and mortality in the context of COVID-19. In this lecture, our recent discoveries on immune-endocrine interactions in the context of infection will be discussed.

Keywords: Viral infection, metabolic disease, COVID-19, CD8 T cells, diabetes mellitus type 2

Multilayer Framework for the Information Spreading Characterization in Social Media during the COVID-19 Crisis

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The understanding of information spreading patterns in social media during the COVID-19 crisis is an important issue of communication related to public health. Before the presence of social media, the distribution of health (and all other) information relied on traditional media, such as newspapers and television. The new era of social media arises new problems in terms of infodemics and misinformation spreading. As stated by the WHO, the COVID-19 outbreak and response culminated with massive infodemics that is dangerous because it makes it difficult for people to find trustworthy sources and reliable guidance when they need it.

In that light, automatic recognition of information spreading patterns may be helpful in the tasks of devising efficient and effective ways to disseminate true and relevant information. In the field of natural language processing (NLP) various approaches have already been defined that can differentiate between various kinds of information, for example, information with a positive or negative attitude; or misinformation vs. mainstream information. However, the COVID-19 crisis brings a completely new realm of challenges in terms of large communication volumes that result in massive datasets, new terminology, new aspects and new specific topics that have come into focus.

The aim of our research is to define a novel framework that can capture a wide set of communication aspects and provide information spreading characterisation. We will perform a quantitative and qualitative analysis on massive datasets based on COVID-19 crisis communication. More precisely, we will propose a multilayer framework that defines a set of approaches, methods and network-based models that capture three aspects of information spreading analysis: (i) content, (ii) context and (iii) dynamic. The content-based analysis of textual information will rely on the various natural language processing methods and approaches for tasks such as keywords/keyphrases extraction, text classification. Additionally, this segment of analysis will include descriptive statistics of the textual information related to COVID-19 crisis communication characteristics. The context-based analysis refers to the analysis of various multilayer network properties on the global, middle and local scale. The analysis of the dynamics involves the analysis of cascade dynamics and other properties such as information trends changing over time.

We expect the proposed approach to provide useful insights that will pave the way to the future development of a system for detecting the spread of misleading and harmful information on social media.

Keywords: information spreading, social networks analysis, natural language processing, COVID-19

Life in the time of COVID-19 - social implications on the security and well-being of vulnerable groups in the European context

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In recent time, humanity has experienced devastating effects of COVID-19 crisis in every segment of social life. The UN Secretary-General António Guterres' statement that COVID-19 pandemic is causing untold human suffering and economic devastation worldwide is, unfortunately, a correct predictor of extent and severity of its effects. While state officials are predominately focused on fighting the pandemic in the medical battlefield, there has been a strong indication that the state reduction of previously attained level of social security rights and services combined with mandatory lockdown measures have severe, unwanted consequences for the most vulnerable members of society. Due to coronavirus mandatory cohabitation, victims of family violence are exposed to higher risk of abuse and deprived from physical space to report the abuser. The risk of child victimisation in respect of mistreatment, abuse, neglect and exploitation is higher than ever, and there is a great probability that criminal offences against the youngest members of society will increase amidst extraordinary circumstances and restrictive measures. Numerous studies have already confirmed that prolonged exposure to criminal acts is a serious risk factor for victims' mental health and well-being, however, the state restrictions within the psychiatric mental health system can be an obstacle to meet victims' needs and to adequately provide mental health services for people with mental difficulties. These who were vulnerable are now more vulnerable than ever and question remains whether the state may bear the COVID-19 social burden and adequately protect them. It is of utter importance to emphasize that scientific community has a moral, ethical and professional obligation to respond to the crisis. The project entitled "Life in the time of COVID-19 - social implications on the security and well-being of vulnerable groups in the European context" is a direct response to this obligation.

Bearing this in mind, the project is planned to critically evaluate different normative techniques that states have used in order to cope with the COVID-19 pandemia and to assess their direct impact on power balance, democratic principal in general and rights of these who are subjected to family violence, struggling with mental issues or are underage and harmed by criminal offences. The project team members will conduct the empirical research at the five police departments centred in Pula, Rijeka, Zagreb, Split and Osijek in order to find out phenomenological and etiological shifts in crime against children and prohibited behaviours related to family violence. The regional distribution will be maintained in the second phase of empirical research dedicated to exploring the experiences of NGO service providers (focus group study) and exposure to family violence of service users (quantitative research with clients of NGO support system). The identical methodological framework will be applied in the assessment of information literacy skills and competences of NGO stuff and their users in the time of pandemics. The empirical phase of the research will be concluded with a study at the University Psychiatric Hospital Vrapče. The obtained theoretical and empirical project outcomes will be compared with the key research findings reached by Slovenian, Italian and Swedish project researchers. The project results will be shared with scientific and professional community, general public, policy makers and persons as-

sociated with vulnerability at research workshops, conferences and science popularisation events and initiatives. By combining different project tactics in interdisciplinary comparative settings, the project research team is strongly determined to reach the final project goal - to enhance the position of vulnerable persons during the state of emergency or quasi-emergency in the European context.

Key words: COVID-19, vulnerability, victims of family violence, child victims of criminal offences, persons with mental difficulties.