# IMMUNOLOGY AT THE CONFLUENCE OF MULTIDISCIPLINARY APPROACHES

# ABSTRACT BOOK

**Institute for Biological Research "Siniša Stanković" National Institute of Republic of Serbia**
**University of Belgrade**

**Immunological Society of Serbia**

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## ABSTRACT BOOK

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# INTRODUCTORY WORDS

Dear colleagues,

It has been our great pleasure to organize this congress, as the first attempt to accelerate interaction between immunologists and other biomedical scientists, as well as between basic and clinical immunologists. We are all witnesses of increased specialization in science. At the same time, there is a growing need for integration of specific fields for the benefit of scientific research. We hope that this congress is a step forward to better integration of biomedical studies.

# PROGRAM

**Friday December 6th**

9:00-9:15 **Nada Pejnović** OPENING WORDS

**OPENING LECTURE** (Nada Pejnović)

9:15-10:00 **Janko Nikolić-Žugić** IMMUNE AGING OF SECONDARY LYMPHOID ORGANS: PERSPECTIVES FOR INTERVENTION

**Session: IMMUNOTHERAPY**

(Đorđe Miljković, Alisa Gruden Movsesijan)

10:00-10:15 **Anica Remenar** ACCELERATE YOUR PACE IN IMMUNE SYSTEM RESEARCH – DURACLONE PANELS, Beckman Coulter/ELTA90MS sponsored lecture

10:15-10:45 **Olivera Finn** TARGETS OF SPONTANEOUS IMMUNOSURVEILLANCE AS VACCINE ANTIGENS FOR CANCER PREVENTION AND THERAPY

10:45-11:10 **Tanja Nikolić** TOLEROGENIC VACCINATION WITH DENDRITIC CELLS IN TYPE 1 DIABETES - FROM AN IDEA TO A TRIAL

11:10-11:30 **Ayca Sayi Yazgan** THE PD-1/PD-L1 IMMUNE INHIBITORY CHECKPOINT IN *H. pylori*- RELATED GASTRIC MALIGNANCIES

11:30-11:45 **Martin Jakopec** AN OVERVIEW OF EVOS IMAGING APPLICATIONS AND SOLUTIONS Thermo Fisher Scientific/Vivogen sponsored lecture

11:45-12:15 **Discussion**

12:15-13:30 *Lunch break/Poster viewing*

**Session: NEXT GENERATION SEQUENCING**
(Ivana Novaković, Sergej Tomić)

| | |
|---|---|
| 13:30-13:45 | **Stefano Romorini** BIO-RAD S3E CELL SORTER: CELL SORTING MADE EASY Labena sponsored lecture |
| 13:45-14:10 | **Borut Peterlin** GENOMIC APPROACH TO MULTIPLE SCLEROSIS |
| 14:10-14:30 | **Ivana Novaković** FROM TRADITIONAL TO MODERN APPROACHES IN ANALYSIS OF DISEASE CAUSING GENES AND GENE VARIANTS: ROLE OF NEXT GENERATION SEQUENCING |
| 14:30-14:50 | **Goran Čuturilo** NEXT GENERATION SEQUENCING AND IMMUNOGENETICS: EXPERIENCE FROM CLINICAL GENETIC SERVICE OF TERCIARY PEDIATRIC REFERRAL CENTRE |
| 14:50-15:05 | **Marko Andabaka** HLA GENOTYPISATION BY NEXT GENERATION SEQUENCING IN PATIENTS WITH NEUROMYELITIS OPTICA SPECTRUM OF DISORDERS |
| 15:05-15:30 | **Discussion** |
| 15:30-16:30 | *Coffee break/Poster session* |

**Session: VASCULITIS**
(Sanvila Rašković, Snežana Aranđelović)

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|---|---|
| 16:00-16:25 | **Sanvila Rašković** NEWS ABOUT ETIOPATHOGENESIS AND THERAPY OF SYSTEMIC LUPUS ERYTHEMATOSUS |
| 16:25-16:50 | **Žikica Jovičić** CAN PRESENCE OF VAROIUS ANTIBODIES PREDILECT CLINICAL MANIFESTATIONS? |
| 16:50-17:15 | **Snežana Aranđelović** CLINICAL AND IMMUNOSEROLOGICAL PHENOTYPES OF CRYOGLOBULINEMIA |
| 17:15-17:30 | **Maja Stojanović** TAKAJASU ARTERITIS- DIAGNOSTIC APPROACH |

17:30-17:45 **Nevena Savić** CRIPTOCCOCUS MENINGOENCEPHALITIS IN DIFFERENTIAL DIAGNOSIS OF NEUROLUPUS-A CASE REPORT

17:45-18:00 **Rada Mišković** STRONGYLOIDASIS AND VASCULITIS COMPLICATED WITH TUBERCULOSIS - A CASE REPORT

18:00-18:30 **Discussion**

**Saturday December 7th**

**PLENARY LECTURES** (Tamara Saksida)

9:15-9:45 **Bojan Polić** DECREASE IN BLOOD GLUCOSE LEVELS DUE TO VIRAL INFECTION PROMOTES INNATE-IMMUNE ANTI-VIRAL RESPONSE

9:45-10:15 **Maja Jagodić** APPLIED OMICS: INSIGHTS INTO THE PATHOGENESIS OF MULTIPLE SCLEROSIS

**Session: METABOLIC INFLAMMATION**

(Ana Đorđević, Nemanja Jovičić)

10:15-10:45 **Rosita Gabbianelli** NUTRIGENOMICS AND INFLAMMATION

10:45-11:05 **Nataša Veličković** IS METAFLAMMATION A USUAL SUSPECT FOR FRUCTOSE-INDUCED METABOLIC DISTURBANCES?

11:05-11:25 **Aleksandra Stanković** THE IMMUNOMODULATORY EFFECTS OF POLYPHENOL RICH JUICE CONSUMPTION ON GENE EXPRESSION IN SUBJECTS AT CVD RISK

11:25-11:45 **Ivana Nikolić** T CELLS AND STRESS – NOVEL REGULATORS OF OBESITY DEVELOPMENT

11:45-12:15 **Discussion**

12:15-13:30 *Lunch break/Poster viewing*

**Session: IMMUNODEFICIENCIES**
(Mario Abinun, Srđan Pašić)

13:30-13:55 **Mario Abinun** AUTOIMMUNITY AND AUTOINFLAMMATION IN PRIMARY IMMUNODEFICIENCY

13:55-14:20 **Desa Lilić** PRIMARY IMMUNE DEFICIENCY AND CANDIDIASIS FROM BENCH TO BEDSIDE

14:20-14:40 **Srđan Pašić** PRIMARY IMMUNODEFICIENCY DISEASES WITH DEFFICIENT ANTIBODY PRODUCTION IN CHILDREN

14:40-15:00 **Slađana Andrejević** THE MANY FACES OF HEREDITARY ANGIOEDEMA

15:00-15:15 **Radovan Miljanović** COMMON VARIABLE IMMUNODEFFICIENCY: 18-YEAR FOLLOW-UP OF PATIENTS IN CLINICAL CENTER OF SERBIA

15:15-15:30 **Discussion**

15:30-16:30 *Coffee break/Poster session*

**Session: NEUROIMMUNOLOGY**
(Irena Lavrnja, Mirjana Nacka-Aleksić)

16:30-17:00 **Diego Centonze** INFLAMMATORY NEURODEGENERATION IN MULTIPLE SCLEROSIS

17:00-17:30 **Jelena Drulović** NEUROFILAMENT LIGHT CHAIN LEVELS AND OLIGOCLONAL IgG BANDS - POTENTIAL BIOMARKERS IN MULTIPLE SCLEROSIS

17:30-18:00 **Gurumoorthy Krishnamoorthy** THE GUT-BRAIN AXIS IN CNS AUTOIMMUNITY

18:00-18:30 **Discussion**

**Sunday December 8th**

**PLENARY LECTURES** (Nada Pejnović)

9:15-9:45 **Vladimir Badovinac** T CELL IMMUNITY DURING SEPSIS-INDUCED IMMUNOPARALYSIS STATE – A FEW SHORT STORIES

9:45-10:15 **Dragana Janković** IMPACT OF INFECTION ON HEMATOPOIESIS AND LYMPHOCYTE HOMEOSTASIS

**Session: PROTEOMICS**

(Marija Gavrović-Jankulović, Marijana Stojanović)

10:15-10:45 **Boris Turk** CYSTEINE CATHEPSINS: FROM SPECIFICITY AND SUBSTRATES TO MINIMALLY INVASIVE DIAGNOSTIC IMAGING IN CANCER

10:45-11:15 **Uta Jappe** NEW ALLERGENS: THEIR IMPACT ON DIAGNOSIS AND TREATMENT AND HOW TO IDENTIFY THEM

11:15-11:30 **Andrijana Nešić** REGULATION OF mRNA EXPRESSION OF TIGHT JUNCTION PROTEINS AND PRO-ALLERGENIC CYTOKINES BY THE MAJOR KIWIFRUIT ALLERGEN ACTINIDIN IN VIVO

11:30-11:45 **Marija Mojić** NKG2D AS A MARKER OF ACTIVE TUMOR-ANTIGEN SPECIFIC $CD8^+$ T CELLS DURING ANTITUMOR IMMUNE RESPONSE IN MOUSE MELANOMA MODEL

11:45-12:15 **Discussion**

12:15-13:30 *Lunch break/Poster viewing*

**Session: AUTOIMMUNITY**

(Miloš Marković, Slavko Mojsilović)

13:30-14:00 **Nebojša Lalić** T CELLS IN TYPE 1 DIABETES: ROLE AND POSSIBILITIES OF MODULATION

14:00-14:30 **Andrey Tchorbanov** NEW APPROACHES FOR SELECTIVE IMMUNOTHERAPY OF AUTOIMMUNE DISEASES BY ENGINEERED CHIMERIC MOLECULES

14:30-14:45 **Alisa Gruden-Movsesijan** TRICHINELLA SPIRALIS PRODUCTS, POWERFUL MODULATORS OF AUTOIMMUNITY

14:45-15:00 **Nemanja Jovičić** EXOGENOUS IL-33 PREVENTS MLD-STZ INDUCTION OF DIABETES AND ATTENUATE INSULITIS IN PREDIABETIC NOD MICE

15:00-15:15 **Ivan Koprivica** ATRA- AND TGF-β-LOADED MICROPARTICLES AMELIORATE TYPE 1 DIABETES IN MICE

15:15-15:30 **Discussion**

15:30-16:30 *Coffee break/Poster session*

**Session: EPIGENETICS**
(Melita Vidaković, Ivana Stojanović)

16:30-17:00 **Marianne Rots** THE PAST, PRESENCE AND FUTURE OF TARGETING EPIGENETIC MARKS: FROM CHEMICALS AND DNA VIA PROTEINS TO RNA AND CLINICAL GENE THERAPY

17:00-17:30 **Tomasz Jurkowski** ERASING EPIGENETIC MEMORY: PRONOUNCED SEQUENCE PREFERENCE OF MAMMALIAN TET ENZYMES GUIDES ACTIVE DNA DEMETHYLATION

17:30-17:45 **Jovan Pešović** VARIANT CCG REPEATS WITHIN *DMPK* EXPANSIONS AND SURROUNDING CpG SITES ARE HETEROGENEOUSLY METHYLATED IN BLOOD CELLS OF MYOTONIC DYSTROPHY TYPE 1 PATIENTS

17:45-18:00 **Katarina Zeljić** THE IMPORTANCE OF STUDYING miRNAS IN ORAL CANCER

18:00-18:15 **Nađa Nikolić** GENE METHYLATION IN HEAD AND NECK TUMORS

18:15-18:30 **Miljana Tanić** APPLICATION OF EPIGENOMICS FOR THE STUDY OF TUMOR HETEROGENEITY IN NON-SMALL-CELL LUNG CANCER

18:30-18:50 **Discussion**

18:50-19:00 **Tamara Saksida** CLOSING WORDS

# ABSTRACTS

Plenary lecture

## IMMUNE AGING OF SECONDARY LYMPHOID ORGANS: PERSPECTIVES FOR INTERVENTION

Janko Nikolich-Žugich

*Department of Immunobiology and the University Arizona Center on Aging, University of Arizona College of Medicine-Tucson, Tucson, AZ, USA*

Adaptive immune system is in charge of precise defense against a highly diverse array of microorganisms. For defense against new infections, the organism deploys naïve, previously antigen-unexposed, T and B lymphocytes, whose antigen-specific receptors recognize, and eventually orchestrate the removal of, the invading microorganisms. Naïve B, and even more so T, lymphocytes numerically diminish with aging. However, new data suggests that those that remain appear to have maintained their functional potential, contrary to an earlier dogma. These findings refocused our attention upon cell-extrinsic defects in immunity with aging. Results will be presented showing that thymic rejuvenation is not sufficient to improve/increase the naïve T cell pool in the secondary lymphoid organs. Moreover, data will be presented showing that aging of lymph nodes, including alteration in their architecture and stromal cell numbers, integrity and function, as well as changes in circulating, most likely soluble, factors, critically modulate both homeostasis and function of the aging immune system. Finally, we will present interventions to improve function in older LN and will discuss implications for immune rejuvenation in humans.

Invited lecture

## TARGETS OF SPONTANEOUS IMMUNOSURVEILLANCE AS VACCINE ANTIGENS FOR CANCER PREVENTION AND THERAPY

Olivera J. Finn

*University of Pittsburgh School of Medicine, Pittsburgh, PA, USA*

Both mutated and non-mutated tumor antigens have been incorporated into vaccines in hope of eliciting or boosting immune responses to control tumor growth. One of the first non-mutated tumor antigens to be reported as a target of human cellular and humoral immunity is MUC1, abnormally expressed on all human adenocarcinomas. Preclinical and clinical studies of therapeutic MUC1 vaccines have been performed in breast, pancreatic, colon, ovarian, prostate and lung cancer. Immune responses were elicited but they were of low titter (antibodies) and low frequency (T cells). Clinical responses were rare. Several reasons for this were elucidated over the years, the major one being the immunosuppressive microenvironment of advanced disease. In studying the expression of the tumor forms of MUC1 we discovered that abnormal expression occurs already on premalignant lesions, and that individuals diagnosed with such lesions, including colonic polyps, benign lung nodules and premalignant pancreatic lesions, generate immunity to abnormal MUC1. This led to our current work testing the MUC1 vaccine in the premalignant setting, in the absence of an established immunosuppressive tumor microenvironment, to induce strong immunity that could prevent premalignant lesions from progressing to cancer. We will report results from two ongoing clinical trials testing a preventative MUC1 vaccine composed of a 100aa peptide from the MUC1 tandem repeat region, the immunogenic portion of the molecule, with the TLR3 agonist Poly-ICLC as adjuvant, in individuals at high risk for developing colon cancer or lung cancer. In this prevention setting the vaccine elicits strong antibody responses as well as strong immune memory, not seen in the therapeutic setting. Antibodies from vaccine responders were cloned and are being developed for antibody-based or CAR-based adoptive T cell therapy

## Friday, December 6th Session: IMMUNOTHERAPY

### Invited lecture

### TOLEROGENIC VACCINATION WITH DENDRITIC CELLS IN TYPE 1 DIABETES
### - FROM AN IDEA TO A TRIAL -

Tanja Nikolić, Vivienne Gibson, S. Laban, A.M. Joosten, J.J. Zwaginga and B.O. Roep

*Department of Immunohematology and Blood Transfusion, LUMC, Leiden, The Netherlands*

Tolerogenic dendritic cells (tolDC) have been nominated as a promising cellular therapy to treat autoimmune conditions. TolDC generated in vitro with vitamin D3 and dexamethason eliminate antigen-specific effector T-cells, promote IL-10 production and generate antigen-specific regulatory T-cells, which in their turn modulate inflammatory dendritic cells and promote tissue-specific infectious tolerance. These findings pointed to tolDC as a conceptually superior approach to induce antigen-specific immunomodulation. To test tolDC in a clinical setting, we designed a study to evaluate the stability and safety in vivo and obtain proof-of-concept evidence of their immunomodulatory properties. First, we evaluated and confirmed the durability of the phenotype and function of clinical-grade tolDCs in vitro. The phenotype and function of tolDCs proved stable despite repeated stimulation with LPS, CD40-L or inflammatory cytokines, specified by expression of high CD52 and low CD86 molecules. To examine whether tolDCs induce antigen-specific tolerance in vivo, HLA-DR4-Tg mice were treated with autologous tolDCs either unpulsed or pulsed with DR4-restricted proinsulin peptide (C19-A3) and monitored for adverse events. One week before the readout, mice were challenged by immunization with intact human proinsulin and autoreactive IL-10 and IFNg responses measured. Vaccination of DR4-Tg mice with tolDCs proved safe and IL-10 production to proinsulin increased after treatment with C19-A3-pulsed tolDCs. Moreover, subsequent immunization of DR4-Tg mice with proinsulin resulted in reduced IFNg production in mice treated with C19-A3-pulsed tolDCs compared to those treated with tolDCs only. Our data show that monocyte modulation with VitD3 and dexamethasone generates potent and stable tolerogenic DCs for clinical cell-therapy. Administration of C19-A3-pulsed tolDCs in humanized mice is safe and shows that tolDCs inhibit autoimmunity in vivo, paving the way for clinical evaluation of the safety and feasibility of intradermal vaccination with proinsulin peptide-pulsed tolDC.

Invited lecture

## THE PD-1/PD-L1 IMMUNE INHIBITORY CHECKPOINT IN *H. pylori*-RELATED GASTRIC MALIGNANCIES

E. Merve Aydin[1,2], Dilan Demir[1,3], Sinem Oktem-Okullu[2], Sawsan S. Said[1], Arzu Tiftikci[4], Ayca Sayi Yazgan[1]

*[1]Molecular Biology-Genetics and Biotechnology Research Center, Istanbul Technical University, Istanbul, Turkey; [2]Department of Medical Microbiology, Acibadem University, Istanbul, Turkey; [3]Research Center for Translational Medicine, Koc University, Istanbul, Turkey; [4]Department of Gastroenterology, Acibadem University, Istanbul, Turkey*

Gastric cancer is one of the most common cancer types worldwide. Even though, early recognition of disease can lead to high survival rates, it is mostly diagnosed in late stages. Infection with *Helicobacter pylori* (*H. pylori*) is the strongest known risk factor for development of gastric cancer. PD-L1 is an immune inhibitory checkpoint protein that is expressed in various cell types including antigen presenting cells and tumor cells. PDL-1 binds to PD-1 (expressed on T-cells) and dampen T-cell activity. This event is crucial for peripheral tolerance and prevent excessive tissue damage. However, it has been suggested that upregulation of PD-L1 favors immune evasion and result in chronic infection with *H. pylori* and development of gastric cancer. Our laboratory focus on investigating the relationship between *H. pylori* and host's immune cells to understand the mechanism of progression of *H.pylori*- induced gastric malignancies such as gastritis and ulcer to gastric cancer. By understanding this mechanism, we aim to contribute to future research on gastric cancer immunotherapies. We are using both mouse infection models and human gastric biopsy samples throughout our studies. During this presentation, I am going to talk about our findings related to the role of PD-1/PD-L1 expressed on regulatory B cells in development of regulatory T cell in a murine setting. Also, I will discuss our data about the expression profile of PD-1 and PD-L1 in human gastritis, ulcer, and gastric cancer patients. Finally, the correlation of PD-L1 and PD-1 with *H. pylori* virulence factors, T-cell subset related cytokine expressions, and clinical outcomes will be emphasized.

Poster presentation

## SHORT TERM FISH OIL TREATMENT ALTERS FATTY ACID COMPOSITION AND CHOLESTEROL-RELATED GENE EXPRESSION IN MOUSE RETINA AND RETINAL PIGMENTED EPITHELIUM (RPE)

Irena Jovanovic Macura[1], Ivana Djuricic[2], Marjana Brkic[1,#], Desanka Milanovic[1], Sladjana Sobajic[2], Selma Kanazir[1] and Sanja Ivkovic[1]

*[1]Institute for Biological research "Sinisa Stankovic", University of Belgrade, Serbia; [2]Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia; [#] current affiliation - Center for the Promotion of Science, Belgrade, Serbia*

The imbalance in omega-6/omega-3 long chain polyunsaturated fatty acids (LC-PUFA) ratio can play a significant role in the development of many chronic and inflammatory diseases, including age related macular degeneration (AMD), the multifactorial disease recently linked to the hypercholesterolemic eye. Fish oil (FO) is a natural source of n-3 long chain polyunsaturated fatty acids (LC-PUFA) and intermittent supplementation of high doses of FO (up to 5g/day for up to 16 weeks) is considered safe for use in human population and is suggested as a prophylaxis for AMD. However, proper eye function depends on the strictly regulated docosahexaenoic fatty acid, DHA (C22:6n3), one of the n-3 LC-PUFAs, and cholesterol homeostasis. As retina/RPE visual system has very specific, and likely different, requirements for cholesterol and DHA homeostasis we analyzed the effects of the FO supplementation on the lipid profile and the expression levels of the genes regulating cholesterol synthesis, transport and elimination in these structures. For the FO treatment, 3 months old B6/SJL mice were divided in two groups – the treated group (n=7) that was supplemented with 100 µl commercial fish oil (DietPharm, FidaFarm Croatia) daily via oral gavage for 21 days, and the control group (n=7), which received the same volume of the water as vehicle. The animals were sacrificed immediately after the treatment, eyes were enucleated, and retinas and RPEs were processed for RNA and fatty acid isolation. Lipid profile analysis and real time RT-PCR showed significant changes in the levels of eicosapentanoic (EPA) n-3 PUFA in retina and RPE, while DHA levels remain unlatered. Furthermore, the FO treatment significantly decreased the expression of the genes regulating cholesterol synthesis and transport in retina. These findings contribute to the better understanding of the role of FO supplementation in the retinal health and function, and can help improve the recommendation strategies.

## Friday, December 6th Session: IMMUNOTHERAPY

Poster presentation

### BENFOTIAMINE DIRECTS DENDRITIC CELLS TOWARD A TOLEROGENIC PHENOTYPE

Iva Božić[1], Neda Đedović[2], Milica Lazarević[2], Đorđe Miljković[2], Irena Lavrnja[1]

*[1]Department of Neurobiology, [2]Department of Immunology, Institute for Biological Research "Siniša Stanković"- National Institute of Republic of Serbia, University of Belgrade*

Dendritic cells (DC) are professional antigen presenting cells that have an important role in inducing the immune response. Under normal conditions, DC reside in peripheral tissues in an immature state. However, they undergo a series of maturation steps in response to inflammatory stimuli. During maturation, DC up-regulate major histocompatibility complex (MHC) class II molecules and co-stimulatory molecules (CD40, CD80, CD86) for antigen presentation and increasingly secrete cytokines. Tolerogenic DC (tolDC) have immunoregulatory properties and are characterized by low expression of MHC class II and co-stimulatory molecules, with limited production of proinflammatory cytokines. TolDC-based immunotherapy is a promising perspective in the treatment of autoimmune diseases. Benfotiamine (S-benzoylthiamine-O-monophosphate) is an S-acyl derivative of vitamin B1 with anti-inflammatory and anti-oxidative properties. Here, we explored the potential of benfotiamine to induce tolerogenic phenotype of DC. DC were cultivated from progenitor bone marrow cells isolated from the femur of C57BL/6 mice. The cells were cultured for 7 days in the presence of granulocyte-macrophage colony-stimulating factor (20 ng/mL) with 100 ng/mL lipopolysaccharide added for the last 24 h of cultivation for maturation. Treatment with benfotiamine (100 μM) was performed on days 0, 2, 4 and 6. FACS analysis showed that benfotiamine applied during differentiation of DC suppressed the expression of MHC class II and CD86, while it did not affect the expression of CD40. The secretion of proinflammatory cytokines TNF, IL-1β, and IL-6 was also decreased. Morphological analysis showed that DC treated with benfotiamine were similar in shape and size to immature DC, despite the maturation stimulus that they were exposed to. The effects of benfotiamine are associated with its suppression of NF-κB translocation to the nucleus. Together, these results show that benfotiamine has the potential to direct DC toward tolDC. Studies on the application of benfotiamine-treated DC in animal models of autoimmunity are warranted.

Poster presentation

## ETHYL PYRUVATE STIMULATES DIFFERENTIATION OF REGULATORY T CELLS *IN VITRO* AND *IN VIVO*

Ivan Koprivica[1], Dragica Gajić[1], Nada Pejnović[1], Tamara Saksida[1], Ivana Stojanović[1]

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Ethyl pyruvate (EP) is a stable form of pyruvate that has shown potent anti-oxidant and anti-inflammatory properties both *in vitro* and *in vivo* and was able to ameliorate systemic inflammation and multiple organ dysfunctions in multiple animal models. Our recent study suggests that the application of EP in the mouse model of type 1 diabetes successfully prevents the clinical manifestation of the disease by augmenting the number of tolerogenic dendritic cells and regulatory T cells (Treg). Our present study indicates that during *in vitro* differentiation of $CD4^+$ naïve cells into Treg, the addition of EP stimulated Treg generation. This was in line with the observed increased proliferation of newly differentiated Treg ($Ki67^+FoxP3^+$). Surprisingly, EP did not scavenge reactive oxygen species (ROS), but rather stimulated ROS production by Treg. In Treg, ROS is mainly generated during oxidative phosphorylation (OXPHOS) during which the majority of energy for the cell is produced. EP probably acted as a substrate in Krebs cycle because the cells produced more pyruvate dehydrogenase, which converts pyruvate to acetyl CoA. EP treatment also resulted in less kinase of pyruvate dehydrogenase, which acts as an inhibitor of Krebs cycle. As a result, there was an evident stimulation of OXPHOS, confirmed by increased ATP production in differentiated Treg. Additionally, EP exerted its stimulatory function on Treg in healthy C57BL/6 mice. When given either intraperitoneally or *per os*, EP increased Treg numbers within the peritoneal cavity or gut-associated lymphoid tissue, respectively. Seemingly, EP promoted differentiation of Treg *in vivo* and did not affect their suppressive properties (proportion of CTLA-$4^+$, $CD39^+$, PD-$1^+$, IL-$10^+$ Treg) or their affinity towards specific effector T helper cells ($ROR\gamma T^+$, $Tbet^+$ or GATA-$3^+$ Treg). In conclusion, EP acts as specific metabolic fuel for Treg generation, likely because these cells mainly rely on OXPHOS-derived energy

Poster presentation

# FIRST OBSERVATION OF EXTRACELLULAR VESICLES FROM MUSCLE LARVAE OF *Trichinella spiralis* AND THEIR IMMUNOMODULATORY PROPERTIES

Maja Kosanović[1], Jelena Cvetković[1], Alisa Gruden-Movsesijan[1], Saša Vasilev[1], Nataša Ilić[1], Ljiljana Sofronić Milosavljević[1]

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Extracellular vesicles (EVs) represent newly discovered, third mode of inter-cellular communication, apart from direct contact and exchanging soluble molecules. They are membrane enclosed vesicles, released from all cells and functioning as transport vehicles between cells, for all types of biomolecules. EVs are involved in all phisyological and pathophysiolologcal processes studied so far, including functioning of immune system. EVs transfer bioinformation between cells of one organism but also between cells of different organisms as in host/parasite system. EVs were found to be produced by several parasites, including helminthes. However, research of helminths' EVs was mostly focused on trematodes, while few data exist on nematodes' EVs and none for *Trichinella spiralis*. Since it was found that some helmintic EVs have immunomodulatory properties, and having in mind that products of *T. spiralis* are known immunomodulators, the question arose if *T. spiralis* produce EVs as part of its excretory-secretory product (ES L1) and weather they have immunomodulatory properties. The EVs were found in ES L1. Transmission electron microscopy revealed round, vesicular structures of 30-80 nm in size. Those vesicles were positive for two out of three glycoproteins with the immunodominant epitope characteristic for muscle larvae of the genus *Trichinella*. Immunomodulatory potential of *T. spiralis* EVs was analyzed in an assay using peripheral blood mononuclear cells (PBMC). PBMC were stimulated with *T. spiralis* EVs and the results showed significantly elevated production of IL10 and IL6, and decresed production of IL-17, compared to control. This indicates that *T. spiralis* EVs can independently induce response similar to that induced by native ES L1.

These results represent first finding of *T. spiralis* muscle larvae EVs and confirmation of their immunomodulatory potential.

Poster presentation

## BISPHOSPHONATED CELLULOSE NANOCRYSTALS INDUCE MATURATION AND ANTI-TUMOR FUNCTIONS OF HUMAN DENDRITIC CELLS VIA GABA-B RECEPTORS

Marina Bekić[1], Miloš Vasiljević[2], Vanja Kokol[3], Marijana Milanović[4], Marija Drakul[2], Dragana Vučević[4], Miodrag Čolić[1,2], Sergej Tomić[1]

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Osteoclast-inhibiting agents, bisphosphonates (BP), recently emerged as potentially promising for the therapy of primary and metastatic bone tumors. The delivery of BP via nanomaterials increases their efficacy and minimizes adverse effects. Accordingly, cellulose nanocrystals (CNC) emerged as attractive new delivery systems with excellent physicochemical properties, biodegradability and biocompatibility. However, anti-tumor properties of BP-CNC conjugates, particularly their effects on the immune response, have not been investigated so far. Dendritic cells (DC) are the key regulators of anti-tumor response. Therefore, we investigated the effects of BP (3-AminoPropylphosphoric Acid (ApA)), either soluble or covalently attached to CNC (CNC-ApA, wt. 7% ApA) on the maturation and anti-tumor functions of human DC *in vitro*. We found that non-toxic doses of CNC-ApA (200 μg/ml), similarly to the effects of higher doses of soluble ApA (70 μg/ml), but not lower ApA doses (14 μg/ml), increased the expression of CD83, CD86, HLA-DR, IL1β, NLRP3, TNF-α, IL-6, IL-10 and IL-12p70, and downregulated the expression CD40, IL-33, ILT-3, ILT-4, PDL1 and CD73 on DC, compared to control, or DC treated with control CNC (w/o ApA). LPS/IFN-γ stimulation further potentiated the effects of higher doses of soluble ApA and CNC-ApA on DC maturation. ApA/CNC-ApA-treated DC increased the relative number of alloreactive Th1 and cytotoxic (Perforin$^+$ Granzyme$^+$) CD4 and CD8 T cells, while decreasing the number of Th17, Th2, Treg and Tr-1 cells, compared to corresponding control DCs. Some of the effects of CNC-ApA (i.e. on CD83, TNF-α, IL-6, CD40, IL33, ILT-3, ILT-4, CD73 expression and downregulation of Th17 and Tregs) were mediated via GABA-B receptor and downstream regulation of cAMP levels in DC, as they could be blocked by a specific GABA-B-R inhibitor, whereas other mechanisms remained unknown. Cumulatively, DC-mediated immunological effects, induced by a new promising BP-CNC nanoplatform, could be useful for the treatment of bone resorptive diseases and cancer.

Poster presentation

## THE CROSSTALK BETWEEN DC-SIGN, TLR2 AND TLR4 TRIGGERS TOLEROGENIC HUMAN DC PHENOTYPE

Natasa Ilic,[1] Jelena Cvetkovic,[1] Alisa Gruden-Movsesijan,[1] Sergej Tomic,[1] Ninoslav Mitic,[1] Elena Pinelli,[2] Ljiljana Sofronic-Milosavljevic[1]

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Human tolerogenic dendritic cells (tolDCs) possess immense potential for specific immunotherapy of autoimmune diseases and our research have recently revealed that ES L1 (*Trichinella spiralis* antigens) treatment of human monocyte-derived DC from healthy donors leads to a stable tolDCs. Underlying molecular events have not been discovered yet. It has been only delineated that TLR2 and TLR4 receptors recognize ES L1 antigens and are requisite for ES L1-induced tolerogenic status of DCs. Cooperation of TLRs with other PRRs may be the mechanism by which these antigens divert pro-inflammatory TLR signaling into anti-inflammatory. As it has been shown that ES L1 antigens possess sugars that could interact with DC-SIGN, this study was dedicated to revealing the role of this receptor on human DCs in binding ES L1 antigens and possible consequential modulation of TLR-signaling pathway. Interaction between ES L1 and DC-SIGN has been proven using recombinant DC-SIGN-Fc in ELISA and Western-blot. To establish the importance of this interaction for immunomodulatory properties of ES L1, immature human monocyte derived DCs were treated with blocking antibodies against DC-SIGN, TLR2 and TLR4 before ES L1 treatment. DC phenotype and their capacity to provoke anti-inflammatory immune response upon co-cultivation with allogeneic T lymphocytes were assessed. Obtained results indicated that simultaneous blocking of the receptors hindered the phenotypic and functional properties of ES L1-treated DCs. Namely, ES L1-induced higher expression of CD40 and production of TGF-β by DCs was impeded. Additionally, blocking of the receptors before ES L1 exposure abolished the ability of DCs to induce Th2 polarization and interfered with the induction of $CD4^{+}CD25^{hi}Foxp3^{+}$ T cells *in vitro.* Overall, these results indicate that DC-SIGN, TLR2 and TLR4 are all immensely involved in the ES L1's induction of tolerogenic characteristics of human monocyte derived DCs. (Grant No: 173047, 175102)

Poster presentation

## *Trichinella* INITIATES THE CROSSTALK BETWEEN DC-SIGN, TLR2 AND TLR4 LEADING TO TOLEROGENIC HUMAN DC PHENOTYPE

Natasa Ilic,[1] Jelena Cvetkovic,[1] Alisa Gruden-Movsesijan,[1] Sergej Tomic,[1] Ninoslav Mitic,[1] Elena Pinelli,[2] Ljiljana Sofronic-Milosavljevic[1]

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Human tolerogenic dendritic cells (tolDCs) possess immense potential for specific immunotherapy of autoimmune diseases. Our research has recently revealed that treatment of human monocyte-derived DC (from healthy donors) with *Trichinella spiralis* muscle larvae ES L1 antigens leads to a stable tolDCs. This study also indicates that TLR2 and TLR4 receptors recognize ES L1 antigens and are requisite for ES L1-induced tolerogenic status of DCs. However, molecular events that underline tolDCs formation under the influence of *T. spiralis*, have not been fully elucidated. It has been shown that ES L1 antigens possess sugars that could interact with DC-SIGN but possible cooperation of TLRs with this PRR was not investigated. The current study was dedicated to revealing the role of DC-SIGN receptor on human DCs in binding ES L1 antigens and possible consequential modulation of TLR-signaling pathway. Interaction between ES L1 and DC-SIGN has been proven using recombinant DC-SIGN-Fc in ELISA and Western-blot. To establish the importance of this interaction for immunomodulatory properties of ES L1, immature human monocyte derived DCs were treated with blocking antibodies against DC-SIGN, TLR2 and TLR4 before ES L1 treatment. DC phenotype and their capacity to provoke anti-inflammatory immune response upon co-cultivation with allogeneic T lymphocytes were assessed. Obtained results indicated that simultaneous blocking of the receptors hindered the phenotypic and functional properties of ES L1-treated DCs. Namely, ES L1-induced higher expression of CD40 and production of TGF-β by DCs was impeded. Additionally, blocking of the receptors before ES L1 exposure abolished the ability of DCs to induce Th2 polarization and interfered with the induction of $CD4^{+}CD25^{hi}Foxp3^{+}$ T cells *in vitro*. Overall, these results indicate that DC-SIGN, TLR2 and TLR4 are all immensely involved in the ES L1's induction of tolerogenic characteristics of human monocyte derived DCs. (Grant No: 173047, 175102)

Poster presentation

# TOLEROGENIC EFFECTS OF ETHYL PYRUVATE ON DENDRITIC CELLS – THE IMPORTANCE OF NRF2 AND NF-KB SIGNALING

Neda Đedović[1*], Đorđe Miljković[1], Irena Lavrnja[2]

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Dendritic cells (DC) are professional antigen presenting cells that are crucial for initiation, propagation, but also for regulation of immune response. Tolerogenic dendritic cells have immuno-regulatory properties and they are a promising potential therapy for multiple sclerosis, as well as for other autoimmune diseases. Ethyl pyruvate (EP) is a redox analogue of dimethyl fumarate (Tecfidera), a drug for multiple sclerosis treatment. We have recently shown that EP has the ability to direct DC towards tolDC in both murine and human DC. In order to investigate mechanisms responsible for EP-imposed tolerance in DC, we examined signal pathways responsible for anti-oxidative cell protection such as Nrf2 signalling pathway, HO-1 and NQO1 enzymes. Furthermore, pro-inflammatory transcription factor NF-κB was also observed. Additionally, change in morphology of DC was assessed via actin filaments staining. EP was applied on days 3 and 6 during 7 days long differentiation of C57BL/6 mouse bone marrow derived immature DC (iDC) or lipopolysaccharide induced mature DC (mDC). Afterwards, immunocitochemistry staining was performed. Results have shown that the maturation of DC led to reduction of the Nrf2 and HO-1 expression, which was successfully prevented by EP. Furthermore, the expression of NQO1 was higher in EP-treated iDC in comparison to untreated iDC. However, the expression in EP treated mDC was lower than in untreated mDC, but still higher than in iDC. Finally, EP-treated mDC had lower expression of NF-κB compared to EP-treated iDC. Moreover, these results are supported by morphological changes of DC after their treatment with EP. While mDC have observable dendrites, EP-treated DC have round shape and are much similar to iDC. In conclusion, these results clearly demonstrate that EP exerts its tolerogenic potential on DC through the up-regulation of anti-oxidative signalling pathways, as well as through the inhibition of pro-inflammatory transcription factor NF-κB.

Poster presentation

# PROSTAGLANDIN E2 POTENTIATE THE SUPPRESSIVE FUNCTIONS OF HUMAN MYELOID DERIVED SUPPRESSOR CELLS AND THEIR CAPACITY TO INDUCE IL-10- PRODUCING REGULATORY T CELLS

Sergej Tomić[1], Bojan Joksimović[2], Marina Bekić[1], Miloš Vasiljević[2], Marijana Milovanović[3], Miodrag Čolić[1,2], Dragana Vučević[3]

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Myeloid-derived suppressor cells (MDSC) emerged as major factors driving the tumor progression and the main obstacle for successful checkpoint blockade therapy. Prostaglandin (PG)E2 is shown critical for the induction of MDSC and their suppressive functions *in vivo*, but it is poorly understood how it affects the capacity of MDSC to induce different subsets of regulatory T cells (Treg). By using a novel protocol for the generation of human mononuclear (M)-MDSC, we showed that PGE2 potentiates the GM-CSF/IL-6-dependent induction of M-MDSC *in vitro*. PGE2 diminished the capacity of GM-CSF/IL-6 M-MDSC to produce proinflammatory cytokines upon activation and augmented their capacity to produce IL-27, IL-33, and TGF-β. These results correlated with an increased potential of PGE2 M-MDSC to suppress T cell proliferation, expand alloreactive Th2 cells, and reduce the development of alloreactive Th17 and cytotoxic CD8 T cells. Interestingly, PGE2 M-MDSC displayed a lower capacity to induce TGF-β-producing $FoxP3^+$ regulatory Tregs compared to GM-CSF/IL-6 M-MDSC which induced $FoxP3^+$ Tregs via IDO-1. In contrast, PGE2 M-MDSC potentiated IL-10 production by $CD8^+$T, Th2, and particularly $CD4^+FoxP3^-$ type 1 Treg (Tr1), the latter of which depended on ILT3 and ILT4 expression. Cumulatively, PGE2 potentiated the suppressive phenotype and functions of human M-MDSC and changed the mechanisms involved in Treg induction, which could be important for investigating new therapeutic strategies focused on MDSC-related effects in tumors and autoimmune diseases with dominant Tr-1-mediated suppression.

Poster presentation

## EFFECTS OF GALECTIN-1 ON IMMUNOMODULATORY PROPERTIES OF HUMAN DENDRITIC CELLS *IN VITRO*

Tanja Džopalić[1], Miloš Kostić[1], Goran Marjanović[1,2], Biljana Božić Nedeljković[3]

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Galectin-1 (Gal-1) has numerous biological functions, such as function in cell survival/apoptosis, regulation of cell cycle, adhesion, and cell migration. The response of dendritic cells (DCs) to pathogenic and inflammatory stimuli is reflected through changes of their phenotype and functions. The aim of our study was to determine the effects of different concentrations of Gal-1 on monocyte-derived (Mo) DCs' maturation and their ability to modulate the immune response *in vitro*. The study was performed in the culture of MoDCs *in vitro*. MoDCs were treated with increasing concentrations of Gal-1 (1, 3 and 6 μg/ml) for 48 h, upon which their phenotypic characteristics, the cytokine profile, and the ability to direct the immune response in the culture with allogeneic $CD4^+$T cells was observed. Gal-1 in all investigated concentrations reduced the expression of CD80 and CD86 molecules on MoDCs compared to their expression on untreated MoDCs. Gal-1 at a concentration of 1 μg/ml and 6 μg/ml led to a significant reduction in IL-12 production, while at a concentration of 3 μg/ml it led to its significant increase. On the other hand, Gal-1 administered in all concentrations induced a significant increase the production of IL-10. Treatment of MoDC with Gal-1 in concentrations of 3 and 6 μg/ml stimulated the production of IL-2 and IFN-γ in the co-culture, while the lowest Gal-1 concentration (1 μg/ml) reduced the production of IL-17 by $CD4^+$T lymphocytes. This study demonstrated a two-part immunomodulatory effect of Gal-1 on MoDC in terms of immunostimulation and immunosuppression, depending on the applied concentration. Future studies require a deeper understanding of the role of Gal-1 in the modulation of phenotypes and MoDC functions depending on the applied concentration in order to better define its role as a potential adjuvant in the preparation of the MoDC for cell therapy.

Invited lecture

## GENOMIC APPROACH TO MULTIPLE SCLEROSIS

Borut Peterlin[1], Lovro Vidmar[1], Jelena Drulović[2], Ivana Novaković[3], Smiljana Ristić[4], Aleš Maver[1]

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Despite extensive research, key aspects of multiple sclerosis etiology and pathogenesis remain unresolved. More than 200 genetic risk loci that have been identified through genome-wide association studies imply the role of many different immune cell types and tissues, including microglia. On the other hand, no convincing rare and penetrant variants have been reported to date, despite familial forms are well recognized. Recently, we described several rare variants in the NLRP1 gene, presumably conveying an increased risk for familial MS. Furthermore, we described the increased burden of rare genetic variation in the inflammasome signaling pathway suggesting its role in the etiology of MS. Finally, we will discuss the potential role of unfixed HERV insertions in the genetic predisposition to MS.

Invited lecture

## FROM TRADITIONAL TO MODERN APPROACHES IN ANALYSIS OF DISEASE CAUSING GENES AND GENE VARIANTS: ROLE OF NEXT GENERATION SEQUENCING

Ivana Novaković

*Neurology Clinic, Faculty of Medicine, University of Belgrade, Belgrade, Serbia*

In the field of inherited disorders, the first important intent is identification of disease causing genes and gene mutations. Traditional approach in detection of genes responsible for some condition includes methods of direct or reverse genetics, such as linkage analysis and positional cloning. Since the late 1980s, in this way have been discovered genes for number of monogenic disorders. In order to detect gene mutations several methods of direct and indirect testing have been developed, with Sanger sequencing as an ultimate step. Over the last two decades Next Generation Sequencing (NGS) changes powerfully strategies mentioned above. NGS comprises group of methods for effective high-throughput DNA sequencing. Different NGS strategies could be used for whole genome or whole exome sequencing, or for analysis of selected gene panels such as “clinical exome” panel. Bionformatic analysis of obtained big data allows analysis of known relevant genes and detection of number of gene variants with in-silico prediction of its significance. In addition, NGS is a powerful method for the identification of new disease causing genes. As an example, we describe our 5-years journey of discovering the PDGFB gene, encoding for beta subunit of platelet derived growth factor, as a new gene responsible for Idiopathic Basal Ganglia Calcification. After initial achievement in large international group, we identified new cases of disease using NGS-based methodology in our laboratory.

Short oral presentation

## NEXT GENERATION SEQUENCING AND IMMUNOGENETICS: EXPERIENCE FROM CLINICAL GENETIC SERVICE OF TERTIARY PEDIATRIC REFERRAL CENTRE

Goran Čuturilo

[1]*Faculty of Medicine, University of Belgrade, Serbia*
[2]*Clinical genetic service, University Children's Hospital, Belgrade, Serbia*

Recently, new technologies have made it possible to obtain a huge number of molecular data for each patient and establish diagnosis in many of them. Clinical genetic service at the University Children's Hospital started to employ modern techniques for exome assessment from November 2014. For multiple-gene assessment we started with solo (patient only) Mendeliome sequencing, followed by subsequent introducing of solo whole-exome sequencing, trio (patient + parents) whole-exome sequencing, and finally genome-sequencing. In terms of chromosomes, traditional and low-resolution analysis of a karyotype has been replaced with chromosomal microarray techniques.
We present the data for pediatric patients who were referred or diagnosed with immunogenetic disorders, such as various types of autoimmune lymphoproliferative syndromes, autoinflamatory disorders, immunodeficinecies. We illustrate diagnostic successes of next generation sequencing techniques, as well as failure or doubtful results in some patients. In summery, next generation sequencing has made it possible to establish diagnosis and provide genetic counseling in a significant proportion of patients and families with immunogenetic disorders.

Short oral presentation

# HLA GENOTYPISATION BY NEXT GENERATION SEQUENCING IN PATIENTS WITH NEUROMYELITIS OPTICA SPECTRUM OF DISORDERS

[1]Marko Andabaka, [2]Marija Branković, [2]Ana Marjanović, [2]Milena Janković, [1,2]Ivana Novaković, [1]Tatjana Pekmezović, [1,2]Jelena Drulović, [1,2]Vladimir Kostić

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Genotypisation of Human Leukocyte Antigen (HLA) locus is important in clinical practice, especially in transplantation and transfusion medicine. In addition, association of HLA genotype with different autoimmune diseases is subject of diagnostic procedures or scientific research. The aim of our study was to analyze HLA genotype in a group of patients with Neuromyelitis Optica spectrum of disorders (NMOSD). Genomic DNA was extracted from peripheral blood samples using salting out method. NGS-based HLA genotyping was performed using Omixon commercial kit for HLA genes A, B, C, DQB1 and DRB1, on Illumina MiSeq NGS platform. For data analysis we used Holotype Twin software by Omixon. This method allowed sequencing of entire coding length, including elements of the 5' and 3' untranslated regions for HLA A, B, C and DQB1, while DRB1 was analyzed from intron 1 to intron 4. In the first run of 24 samples we obtained 9 completed HLA genotypes. In these cases, all 5 analyzed genes were characterized at 8-digit level. In the remaining 15 samples partial HLA genotype was determined, mainly on the 6-digit level for genes HLA A, B and DQB1, while genes HLA C and DRB1 showed ambiguous results. Our data confirmed association of particular HLA DQB1 alleles with NMO observed in literature. Further investigation of larger group of patients is necessary. From technical aspect, we suggest importance of quality control in all steps of NGS protocol, from genomic DNA preparation, via HLA amplification, to library preparation and quantification.

## Invited lecture
## NEWS ABOUT ETIOPATHOGENESIS AND THERAPY OF SYSTEMIC LUPUS ERYTHEMATOSUS

Sanvila Rašković

*Medical Faculty, Belgrade, Clinics for Allergology and Immunology KCS*

The interaction between exposome, its relations to genome and the subsequent modifications of the genome, reffered as epigenome, are potential pathway for SLE onset and progression. Epigenetic changes could be: altered DNA methylation, histone modification, non coding RNAs. In SLE, hypomethylation of CD4+ T cells, and in B cells is demonstrated. Monozygot twins have only 24% concordace rate with SLE. Particulate matter measuring 2,5 microns and smaller are associated with prevalence of SLE. Trace elements: uranium, lead and cadmium are capable to induce autoimmunity and increase a production of ANA. Silica and asbestos exposure also promote autoimmunity. Exogenous estrogen administration increase a risk for SLE. The role of xenoestrogen, such as bisphenol A, is implicated in increasing of Ab prodution. UVA radiation 320-400nm act on keratinocytes and produce DNA damage.

Organ specific biomarkers in lupus nephritis are: renal biopsy, proteinuria, ds DNA, C3, C4, also genetic markers: MCP-1 polymorphism, ITGAM gene , levels of ACE. DNA methylation biomarkers are: circulating micro RNAs, APRIL. Biomarkers for NPSLE in CSF: Il-6, Il-8, Il-10, TNF alfa, ACA, U1RNP. For cutaneous manifestations in SEL: antiannexin 1.

Infection is a common problem in patients with SLE. Infection can mimic exacerbations of SLE. We use conventional biomarkers for infection such as CRP - most sensitive and specific marker for bacterial infections in SEL. Procalcitonin is a promising marker for respiratory bacterial infection, sometimes is complicated to differentiate between sepsis and SLE flare. Hematuria and piuria may present a clinical dilemma. The expression of CD64 on the surface of PMN increases in response to microbial wall components, so CD64 could be a useful biomarker. Also, CD27 high plasma cells freqency correlates with SLEDAI and some autoantibodies.

## Invited lecture
## CLINICAL AND IMMUNOSEROLOGICAL PHENOTYPES OF CRYOGLOBULINEMIA

Snežana Aranđelović

*Clinic for Allergology and Immunology Clinical Centre of Serbia*
*Medical Faculty, University of Belgrade*

Cryoglobulins are serum immunoglobulins that precipitate at temperatures lower than $37^0$C and redissolve after rewarming. Their occurrence is most often associated with various infections, autoimmune and malignant disorders, or the cause of it remains obscure (essential cryoglobulinemia (EMC). Three basic types are recognized according to the clonality and type of immunoglobulins. Type I consists of monoclonal immunoglobulins (IG), while in Type II cryoglobulins are a mixture of monoclonal IgM and polyclonal IgG. Type three cryoglobulis are mixture polyclonal IG. Types II and III are referred to as mixed mixed cryoglobulinemias (MC). MC deposits of immune complexes in walls of blood vessels might lead to vasculitis of small and medium blood vessels, giving the histological picture of leukocytoclastic vasculitis. Clinically, this inflammation is manifested as arthralgias, fatigue, and lesions mainly in skin, kidneys and peripheral nerves, however, any organ system can be involved.
Most of the so far reported clinical and laboratory investigations deal with mixed CG in chronic hepatitis C virus (HCV) infection while the data concerning essential CG vasculitis (CV) are scarce. Only recently, so-called noninfectious CV has attracted more attention of the medical community. Chronic antigenic stimulation, increased cytokine and growth factor (BLyS) levels and complement activation may are implicated in the pathogenesis of CV, etiology of which remains largely unknown.
Cryoglobulinemia is not a unique entity in either clinical or laboratory terms. The amount and type of cryoglobulin, the presence of viral markers, autoantibodies, complement consumption, and cytokine concentrations may be important in the onset, development, and severity of the clinical picture of cryoglobulinemic vasculitis. Increased B lymphocyte proliferation may play a pathogenic role in a number of patients with cryoglobulinemia. It is possible to define subgroups (phenotypes) of patients with cryoglobulinemia who have different disease evolution and prognosis.

Short oral presentation

## RECENT ADVANCES IN THE DIAGNOSIS AND ASSESSMENT OF TAKAYASU ARTERITIS

Maja Stojanovic[1], Zorana Andric[2], Vladimir Milivojevic[3], Ivan Rankovic[4], Marija Stankovic[5], Rada Miskovic[1], Dragana Jovanovic[6], Aleksandra Peric Popadic[1], Jasna Bolpacic[1], Dusan Popadic[7], Sanvila Raskovic[1]

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Takayasu arteritis (TA) is a rare, systemic vasculitis that predominanatly affects the aorta, its major branches and pulmonary arteries. While our understanding of the pathogenesis of TA has considerably improved during the last two decades, the exact pathogenic mechanism remains to be elucidated. Historically, cell-mediated autoimmunity has been strongly implicated in the pathogenesis of TA. Hystopathological investigations would be very useful, but in majority of cases we lack samples to be analyzed. Immunohistochemical studies of infiltrating cells from the aortic tissue of patients with TA have showed infiltration by macrophages, natural killer (NK) cells, neutrophils, CD4+ T cells, CD8+ T cells and γδ T cells. Based on this finding, novel imaging techniques such as 18F- fluorodeoxyglucose position emission tomographies (PET) computed tomography (CT) and PET magnetic resonance (MR) angiography allow us, not only to visualize inflammation by showing the hypermetabolic infiltrate, but also to better characterize and quantify disease activity.As we have known that certain genetic factors play an important role in the development of TA, we aimed to study the association between human leukocyte antigen (HLA) and TA susceptibility. The strong association between HLA-B*52 and TA in the Serbian TA cohort was found. The HLA-A*32, -B*15 and -B*57 allelic groups and DRB1*15:02-DQB1*05 haplotype, as susceptibility factors, and HLA-C*03, described for the first time in the literature as a protective allelic group in TA patients, still need to be confirmed in a larger study population. Searching for a non-invasive test, we have recently identified ELF, a biochemical test consisted of three serum biomarkers: hyaluronic acid, tissue inhibitors of metalloproteinases and amino-terminal propeptide of procollagen type III, as a potential promissing tool for disease assesment. The performance and considerable diagnostic value for the prediction of disease progression due to vessel wall fibrosis still remain to be explored in future studies.

Short oral presentation

## CRYPTOCOCCAL MENINGOENCEPHALITIS AS A DIFFERENTIAL DIAGNOSIS OF NEUROLUPUS - CASE REPORT

Nevena Savic [1], Nikola Mitrovic [2, 3], Aleksandra Peric Popadic [1, 2], Vesna Tomic Spiric [1, 2], Sanvila Raskovic [1, 2]

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A.A. born in 1991, was hospitalized at the KCS Allergology and Immunology Clinic on suspicion of having SEL. Four months before that, she had an infectious syndrome. Ten days later, she was hospitalized for shortness of breath, bilateral pleural effusion, lower leg swelling. The renal lesion was verified: elevated values of nitrogenous substances, significant proteinuria and pathological urine sediment. It was concluded that this was a renal weakness initially presented with hypervolemia, hypertension, and signs of cardiac decompensation. A kidney biopsy showed severe damage that almost matched the end stage kidney disease in the RPGN field. It has been decided to begin immunosuppressive prednisolone therapy of 1mg / kg tt. After a positive ANA titer, hospitalization at the Allergology and Immunology Clinic is scheduled. When she was admitted to the clinic, she reported headache, diplopia and vomiting. The asymmetric rhyme of the oculi, convergent strabismus and ptosis of the left eye were noted in the objective finding. Meningeal signs were negative; CT of the endocranium was neat. Neurological findings suggested neurolupus. Endocranial MR and diplopia test were advised. In consultation with the infectologist, an LP Cryptococcus shower was done, the preliminary finding was positive (a lot of cryptococci were found). The patient was transferred to the Infectious Diseases Clinic.

Nervous system involvement is present in 50% of patients with SEL. American rheumatologists have identified 19 neuropsychiatric syndromes that can be divided into manifestations of the central and peripheral nervous systems. In 40% of patients, CNS damage is due to the disease itself, in others it is due to infections, metabolic disorders, and drug side effects. Since these are patients belonging to the group of immunocompromised patients with immunodeficiency, caused by long-term use of corticosteroid therapy and / or cytostatics, differential opportunistic infections must always be considered.

Short oral presentation

## STRONGYLOIDIASIS AND TUBERCULOSIS IN A PATIENT WITH SYSTEMIC VASCULITIS – CASE REPORT

Rada Miskovic [1 2], Aleksandra Plavsic[1 2], Maja Stojanovic[1 2], Aleksandra Peric Popadic[1 2], Sanvila Raskovic[1 2]

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*S. stercoralis* infection can be clinically silent for decades. However, in immunosuppressed patients, especially those on corticosteroid therapy, hyperinfective syndrome and disseminated strongyloidiasis can occur. Corticosteroid therapy is also, a risk factor for the development of active tuberculosis.

We report the case of hyperinfective syndrome complicated by the development of bacterial meningitis and tuberculosis in a patient with polyarteritis nodosa treated with corticosteroids. The diagnosis of strongyloidiasis was made by finding of larvae and adult parasites in the samples of the upper gastrointestinal tract mucosa and the stool. Eradication of *S. stercoralis* was achieved by subcutaneous administration of the veterinary formulation of ivermectin. The clinical course was complicated by the development of tuberculous lymphadenitis and pulmonary tuberculosis. Despite the use of tuberculostatic therapy and supportive measures, a lethal outcome occurred.

Clinical manifestations of strongyloidiasis in patients with systemic vasculitis often resemble an exacerbation of the underlying disease, remaining unrecognized for a long time. The use of immunosuppressive therapy in these patients increases, not only the risk of more severe forms of strongyloidiasis, but also the risk of developing tuberculosis. A specific interrelation between strongyloidiasis and tuberculosis has been suggested, possibly through the phenomenon of helminth-induced imunossupression.

Poster presentation

## HYPERSENSITIVE VASCULITIS AS A CONSEQUENCE OF TREPONEMA PALLIDUM INFECTION - CASE REPORT

Aleksandra Peric-Popadic

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We describe a case of a 20-year-old man diagnosed with hypersensitive vasculitis most likely caused by Treponema pallida infection. The disease began predominantly with changes in the lower legs, of the type of numerous disseminated palpable purpura, partially fused into purplish plaques with single erythematous maculae on the forearms with pain in the feet and right knee. Local therapy with anti-inflammatory creams and antihistamines did not help. One month before the onset of the changes, he was cold with symptoms: nasal secretions, coughing without fever, pain in the right ear, diarrhea, fresh blood in the stool. He took Ibuprofen from his medication. In clinical presentation, changes in purpura skin, in laboratory analyzes polyclonal stimulation with elevated IgG, IgA, ANA positivity. Other analyzes: positive calprotectin, positive VDRL, in later course extremely high TPHA, ACLA IgG and IgM class, positive LA, pathological urine sediment, erythrocyturia, hyaline cylinders, proteinuria. Based on the clinical presentation of skin changes of the purpura type, PH-proven small blood vessel vasculitis, calprotectin and its normalization to the applied corticosteroid therapy, renal lesions with nephrotic grade proteinuria (8.5 gr for 24 h), the diagnosis of Henoch-Schonlein purpura was made. The patient was treated with intensive corticosteroid therapy 1mg / kg TT as well as "pulse" therapies of corticosteroids and three "pulses" of cyclophosphamide 800 mg per month, when proteinuria was reduced to 3g ... and then 0.25 gr for 24 hours. On the basis of positive treponemal and non-treponemal tests on Treponema Ag, he was diagnosed as Lues latens tarda and, in consultation with an infectologist and dermatovenerologist, pencillin preparations Benzatin pecinillin were administered according to the protocol, with a decrease in VDRL and TPHA parameters. He was discharged in clinical remission with Pronison 5mg therapy, mycophenolate mofetil 2X500mg, IPP, as well as treatment advice for all sexual partners.

## Poster presentation
## CASE REPORT FOR EOSINOPHILIC GRAULOMATOSIS WITH POLYANGINITIS

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Churg Strauss syndrome or allergic eosinophilic graulomatosis with polyanginitis is a very rare form of vasculitis. In this disease, there are signs of vasculitis on the skin and internal organs associated with asthma and increased eosinophils. The disease can be caused by a variety of hereditary and external factors. This syndrome usually occurs in long-standing asthma and in patients with familial atopic predisposition. In 1990, the American Association of Rheumatologists (ACR) set 6 criteria for the diagnosis of Churg Strauss sydrome (CSS): 1. Asthma in 97% of patients; 2. Eosinophilia> 10% in peripheral blood; 3. Paranasal sinusitis (90%), often nasal polyps (61%); 4. Infiltrates in the lungs that migrate or are transient (26-77%); 5. Peripheral neuropathy; 6. Histological findings of perivascular eosinophilic infiltrates or granulomas, which are vasculitis of small and medium sized blood vessels, arteries and veins. A 33-year-old patient has been treated for asthma since year 2010. 7-8 years ago, he had sinus surgery. In June 2018., gallbladder surgery is done. 10 days after surgery, he begins to fever and develop bilateral pneumonia and wound infection in which Staphylococcus foecalis is isolated. He continues to fever for the next 32 days regardless of antibiotic therapy and he is been hospitalized at the Infectious Diseases Clinic. After treatment at the Infectious Disease Clinic, fever has stoped, but he was still in poor general condition. Two days after discharge from the Infectious Disease Clinic, the condition in terms of heart failure worsens. The patient is hospitalized at the Clinic for Diseases of the Heart, Blood Vessels and Rheumatism, where complete cardiac and laboratory tests are performed. In the laboratory, eosinophilia up to 60%, is observed. Immunoassay is done with ANA: negative, ENEA6 profile: negative, PANCA: neg., PANCA: neg. Ultrasound of the heart shows dilated cardiomyopathy EF: 18%, with severe insufficiency of the mitral valve, and moderate pulmonary hypertension, as well as signs of pericardial and pleural effusion. Glucocorticoid therapy is administered. The pulmonologist and the infectologist completely rule out the disease in terms of myocarditis and possible processes in the lungs, and the pulomolgist confirms previously verified asthma bronchialae. A medical review board of immunologists, rheumatologists, pulmonologists, infectologists, cardiologists concludes the diagnosis of Churg Strauss syndrome on the basis of the clinical picture and laboratory parameters. The findings are summarized on a cardiac surgery board indicating cardiac transplantation.

Poster presentation

## CORRELATION BETWEEN GUT MICROBIOTA COMPOSITION AND THERAPEUTIC POTENTIAL OF MYELOID-DERIVED SUPPRESSOR CELLS IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Gut microbiota composition is increasingly recognized as a critical factor determining the efficacy of different immunotherapeutic approaches. However, it is not known how it correlates with a therapeutic potential of myeloid-derived suppressor cells (MDSC), key suppressors in various autoimmune diseases (AD). Therefore, we investigated how the potential of MDSC-therapy to suppress symptoms of multiple sclerosis (MS) correlate with gut microbiota composition. Autologous MDSC generated from bone marrow cells were applied as single therapy to spinal cord homogenate/CFA-induced experimental autoimmune encephalomyelitis (EAE) in Dark Agouti (DA) rats, an animal model of MS. MDSC-therapy resulted in a significant attenuation of EAE symptoms over 30 days of disease monitoring. These results correlated with lower percentage of proinflammatory interferon-gamma and interleukin-17 producing cells and higher percentage of antiinflammatory IL-10 producing cells in spinal cord and spleen. The effects of EAE and MDSC-therapy on gut microbial composition were studied using 16S rDNA-based metagenomic analyses of fecal samples collected prior to the induction of EAE, and at the peak of the disease. The induction of EAE in control group of animals, resulted in a lower microbiota diversity compared to healthy controls, whereas the MDSC therapy preserved the microbial diversity in treated animals. In addition, the induction of EAE in control group correlated with a higher relative abundance of *Peptococcaceae*, whose role in the chronic-progressive course of MS was previously reported. Besides, the relative abundance of *Veillonellaceae*, known to produce immunosuppressive short chain fatty acid (SCFA), was significantly decreased in the control EAE group. In contrast, MDSC therapy resulted in the preservation of *Veillonellaceae* and elevated *Ruminococcaceae*, another SCFA-producing family and *Coriobacteriaceae,* which are known to produce immunosuppressive metabolites from phytoestrogens. Based on these results, MDSC therapy and the observed changes in microbial diversity could be further developed as a promising therapy for MS and other AD.

Poster presentation

## VARIABILITY OF GUT AND LUNG MICROBIOTA IN RAT STRAINS KNOWN TO DIFFER IN REACTIVITY TO IMMUNE STIMULY

Dušanka Popović[1,2], Aleksandra Popov Aleksandrov[1], Dina Tucović[1], Jelena Kulaš[1], Milica Zeljković[1], Amarela Terzić–Vidojević[2], Jovanka Lukić[2], Nataša Golić[2], Ivana Mirkov[1], Maja Tolinački[2]

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The gut microbiome is very important for hosts' proper essential functions, as microbiota influence both near and distant organs and disruption at any level of this complex relation can be underlying cause for many diseases. Both intestinal bacterial population and their metabolic products are involved in immune system development, activity and maintenance of immune homeostasis. Previous findings revealed differences in immune reactivity in Albino Oxford (AO) and Dark Agouti (DA) rat strains in various diseases models (such as experimental autoimmune encephalomyelitis, rheumatoid arthritis, contact hypersensitivity reaction, pulmonary aspergillosis etc.), and in response to xenobiotics such as cadmium, warfarin, etc.

The aim of present study was to investigate microbial composition of gut (duodenum, jejunum, coecum and colon) and lungs of AO and DA rats using DGGE method. Although similar number of bacterial species were noted in both tissue and lumen of duodenum, bacterial composition differ between these two strains (solely 76.2% and 44.4% species were common in both strains tissue and lumen, respectively), while greater variability was noted in DA rats. Similar results were noted in jejunum. In contrast to duodenum and jejunum, higher number of bacterial species were detected in coecum (content) and colon (tissue and content) of AO rats. Around 50% of detected bacteria were present in both gut segments of both strains. Analysis of DGGE bands obtained from lung tissue revealed similar number of bacteria in both strains, but solely 54.5% were common. Further investigations will be directed to identification of bacterial species and try to connect observed differences in microbial composition with different immune reactivity in AO and DA rats.

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Poster presentation

## PROTECTIVE ROLE OF EXOPOLYSACCHARIDES PRODUCED BY *Lactobacillus plantarum* BGAN8 AGAINST CADMIUM INDUCED DAMAGE ON Caco-2 CELLS

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One of the most toxic heavy metals is cadmium which was classified as a human carcinogen. Cadmium is capable of induction of numerous acute and chronic disorders. Several studies have shown that inflammation and oxidative stress play a key role in the toxicity and carcinogenicity. Some reports have shown that species belonging to *Lactobacillus* sp. present in the human gastrointestinal tract and in fermented foods have the ability to bind and detoxify heavy metal ions. There are evidences that exopolysaccharides (EPS) can be used as tools for removing heavy metal ions by detoxication, which prevents their absorption from the gastrointestinal tract by host cells.

*Lactobacillus plantarum* BGAN8 showed the best cadmium binding ability, among others EPS producing strains from Laboratory for Molecular Microbiology collection (LMM collection). So, the aim of this study was to determine the role of isolated and purified EPS from *Lactobacillus plantarum* BGAN8 (EPS-AN8) in protection of cadmium induced damage like inflammation and oxidative stress in Caco-2 cells.

The subtoxic dose of $CdCl_2$ (the concentration that has killed less than 10% of Caco-2 cells) was determined by LDH and MTT assay. Caco-2 cells were treated with subtoxic dose of $CdCl_2$ and EPS-AN8. The changes in expression of target genes were determined by quantitative PCR, while the changes in permeability of Caco-2 cells were tracked by lucifer yellow assay.

Treatment of differentiated $CdCl_2$ treated Caco-2 cells monolayer with EPS-AN8 alleviated inflammation and oxidative stress (decreased level of expression of mRNA *IL-8, NQO1* genes) and also decreased level of lucifer yellow flux. In conclusion, EPS-AN8 might be used as postbiotic in prevention and treatment of cadmium-induced damages.

Poster presentation

## PROBIOTIC ACTIVITY AND MICROENCAPSULATION OF Lactobacillus reuteri B2 ISOLATED FROM FECES OF C57BL/6 MICE

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The major parts of the commensal microbial flora of the human gastrointestinal tract (GIT) belong to the lactic acid bacteria (LAB) that are frequently used as probiotics. Probiotics must survive passage through the upper GIT and reach its site of action alive, where they have to maintain their stability, viability and function. Preparation of various biopolymer-based carriers and microencapsulation become inevitable part of probiotics-focused researches. In this study, we assessed probiotic activity of *Lactobacillus reuteri* B2 selected from the panel of LAB isolated from the feces of C57BL/6 mice. L. reuteri B2 was evaluated as potential probiotic and its microencapsulation with alginate-based materials were performed. We hypothesized that if *L. reuteri* B2 in the free form can survive all conditions in the GIT then the usage of the appropriate biomaterials for microencapsulation would improve its viability and stability in GIT. Consequently, there has been assessed *L. reuteri* B2 in free and in microencapsulated form, in vitro, in the culture of epithelial cells. High survival rate of *L. reuteri* B2 at low pH (2.0- 4.0) and in the presence of the bile salts at concentrations up to 0.30% imply that it can survive harsh conditions within GIT. Likewise, *L. reuteri* B2 strong antimicrobial activity toward pathogen species on which this strain has been assessed. Furthermore, testing of the alginate-based polymers revealed no negative impact on the viability of epithelial cells. Results obtained from this study highly encourage further research on the impact of alginate- encapsulated L. reuteri B2 in physiological conditions as well as for the prevention or treatment of some pathological states. Additionally, demonstrating extended viability of encapsulated probiotics in vivo will justify scaling up the encapsulation process for commercial application.

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Poster presentation

## THE EFFECTS OF HEAT-KILLED *Enterococcus faecium* BGPAS1-3 ON EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS IN DARK AGOUTI RATS

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Experimental autoimmune encephalomyelitis (EAE) is an animal model of multiple sclerosis (MS), a chronic inflammatory disease of the central nervous system (CNS). Recent evidence suggests the involvement of gut microbiota dysbiosis in etiology of MS. Hence, the modification of gut microbiota composition could be an effective approach in MS prevention/treatment. The aim of this study was to evaluate the effect of the heat-killed cells of *Enterococcus faecium* BGPAS1-3 on gut microbiota composition and EAE development in female DA rats. The results showed that the oral application of the heat-killed BGPAS1-3 during 30 days alleviated the clinical symptoms of the disease. The beneficial effects were paralleled with less immune cells infiltrating the CNS, judged by histological examination, as well as their limited ability to produce the major inflammatory cytokines interferon-gamma and interleukin-17, as deduced by ELISA. The effect of heat-killed BGPAS1-3 cells on the gut microbiota was examined by Denaturing Gradient Gel Electrophoresis (DGGE) and Next-Generation Sequencing (NGS) analysis. Based on DGGE analysis, *Shuttleworthia satelles* appeared only in feces sampled from control animals 30 days after EAE induction. *Lactobacillus murinus* and *En. hirae* were present only in feces of immunized animals that were treated for 30 days with heat-killed BGPAS1-3, although not in the control group. Additionally, the results of NGS analysis revealed that some members of *Firmicutes, Bacteroidetes, and Proteobacteria* were more prevalent in feces of immunized animals treated with heat-killed BGPAS1-3 than in control groups. The changes in gut microbiota were mirrored in lower cellularity and reduced the proportion of interleukin-17-producing cells in mesenteric lymph nodes. The obtained results indicate that oral administration of BGPAS1-3 leads to modulation of the autoimmune response, modification of gut microbiota composition and eventually to the alleviation of the disease symptoms.

Poster presentation

## SPECIFICITY OF IgA ISOTYPES IS DEFINED BY THE LOCATION AND THE NATURE OF BACTERIAL ANTIGENIC DETERMINANTS

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The breakthrough in microbiota analysis calls for the thorough analysis of the steady-state antibody reactivity to different microorganisms. The location of the antibodies plays a key role in determining host-microorganism relationship. Here we compared the level of specificity of serum and salivary IgA antibodies towards different bacterial antigens. Enzyme-linked immunosorbent assays targeting four designated antigens – whole *Escherichia coli* cells - urinary infection clinical isolate, whole *Lactobacillus rhamnosus* LA68 cells, *Escherichia coli* O55:B5 lipopolysaccharide (LPS) and *Staphylococcus aureus* peptidoglycan (PGN) were performed. The tested population comprised 14 healthy adults with serum and saliva samples collected at the same time. Both total IgA and IgA subclasses were analyzed and compared. The selected dilutions of serum and saliva used were such that there were effectively 10 x more serum antibodies than salivary ones in the assay. Notwithstanding, extremely high correlations in salivary IgA were obtained between very distant bacterial species such as *L. rhamnosus* and *E. coli*, as well as towards isolated components of microorganisms ($r$ = 0.7-0.96), implying high cross-reactivity and/or low specificity. For serum IgA, the reactivity was profoundly different. Serum IgA differentiates G + from G- microorganisms, i.e. serum IgA towards *E. coli* correlated best with serum IgA towards LPS (0.86), and serum IgA against *L. rhamnosus* correlated best with the anti-PGN IgA2 response (0.88).

Serum IgA response against *E. coli* cells consisted primarily of IgA1 response, whereas serum response to *L. rhamnosus* cells consisted primarily of IgA2 response. For isolated components, serum IgA against LPS was guided by IgA2 response and towards PNG by IgA1 response. The explanation of this phenomenon exceeds the capacity of this study and confirms the delicacy, specificity and orchestration of the immune system.

Poster presentation

## GABA – PRODUCING *Lactobacillus brevis* BGZLS10-17 STRENGTHENS GUT EPITHELIAL BARRIER AND REDUCES INFLAMMATION *IN VITRO*

Svetlana Soković Bajić[1], Jelena Đokić[1], Miroslav Dinić[1], Sergej Tomić[2], Emilija Brdarić[1], Natasa Golić[1], Maja Tolinački[1]

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The characterization of molecules and mechanisms in the bases of probiotic effects of bacteria is necessary for their safe and efficient application in various pathophysiological conditions. GABA-producing Lactic Acid Bacteria, such as *Lactobacillus brevis* BGZLS10-17, isolated from dairy product, are considered as a valuable source of naturally produced GABA as this molecule have wide range of physiological effects. In that mean the aim of this study was to evaluate the effects of GABA containing bacterial cell-free supernatant obtained from BGZLS10-17 culture on intestinal epithelial barrier (differentiated Caco-2 cells monolayer) and immune cells (mesenteric lymph node cells, MLNC) exposed to inflammatory conditions *in vitro*. The treatment of Caco-2 with IL-1β induced the expression of mRNA for pro-inflammatory IL-8 cytokine while decreased the expression of mRNA for tight junction proteins and TGF-β thus mimicking deleterious effects of inflammation on intestinal epithelial barrier. Importantly, BGZLS10-17 supernatant containing GABA alleviated these effects by attenuating IL-8 production and preserving the tight junction proteins and TGF-β expression in Caco-2 exposed to IL-1β. Further, we investigated the effects of GABA containing BGZLS10-17 supernatant on Concanavalin A-stimulated MLNC. We have shown that GABA containing supernatant have strong immunosuppressive effect by inhibiting proliferation of MLNC, the production of IFN-γ and IL-17 and the expression of MHCII and CD80 by these cells. At the other hand, GABA containing supernatant exposed strong stimulatory effect on the expression of immunosuppressive molecules such as Foxp3+, IL-10, TGF-β, CTLA4 and SIRPα in MLNC. Considering all these results, we assume that GABA-producing BGZLS10-17 could have significant role in the maintenance of intestinal epithelial barrier integrity and immune system homeostasis when used as supplement to therapy of diseases related to exacerbated inflammation.

Poster presentation

## POTENTIAL IMPACT OF EARLY-LIFE PROBIOTIC SUPPLEMENTATION ON PERITONEAL MACROPHAGE FUNCTION

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Clinical and animal trials show that early life probiotic consumption provides health benefits in adult life by modulating the immune response. We tested the effects of early life oral consumption of the probiotic *Lactobacillus rhamnosus* on the function and phenotype of rat peritoneal cavity cells in a model of induced colitis. For the first month of their lives, rats were either fed with an aqueous probiotic bacteria suspension (LB group) or tap water (control group). When the rats grew to 3 months old, we studied the response of their peritoneal macrophages to autologous fecal bacteria stimulation *in vitro*, both before and after colitis induction (TNBS 40mg/kg of body mass in 50% ethanol). Compared to the controls, the peritoneal cavity cells of the LB group produced less nitric oxide (NO) and had an increased proportion of CD163+ cells. The rats in the LB group have shown milder symptoms of colitis (shorter length of colon under necrosis, less severe submucosal infiltration, lesser degree of colonic wall thickening), along with a diminished increase of peritoneal pro-inflammatory CCR7+ cells and blunted NO production in response to stimulation by autologous fecal bacteria. Our results may indicate that early oral probiotic administration attenuates macrophage responses to fecal bacteria, which are the primary cause of tissue inflammation and necrosis in chemically induced colitis models, and that this attenuation may be involved in improving the health of colitic rats. (Supported by Ministry of Education, Science and Technological development, Republic of Serbia, Grant No 175050).

Poster presentation

## NEUROPROTECTIVE ROLE OF SELECTED ANTIOXIDANT AGENTS IN PREVENTING CISPLATIN-INDUCED DAMAGE OF HUMAN NEURONS *IN VITRO*

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Chemotherapy-induced peripheral neuropathy (CIPN) is a side effect of platinum-based chemotherapy and decreases the quality of life of cancer patients. We compared neuroprotective properties of several agents using an *in vitro* model of terminally differentiated human cells NT2-N derived from cell line NT2/D1. Sodium azide and an active metabolite of amifostine (WR1065) increase cell viability in simultaneous treatment with cisplatin. In addition, WR1065 protects the non-dividing neurons by decreasing cisplatin caused oxidative stress and apoptosis. Accumulation of Pt in cisplatin-treated cells was heterogeneous, but the frequency and concentration of Pt in cells were lowered in the presence of WR1065 as shown by X-ray fluorescence microscopy (XFM). Transition metals accumulation accompanied Pt increase in cells; this effect was equally diminished in the presence of WR1065. To analyze possible chemical modulation of Pt-DNA bonds, we examined the platinum LIII near edge spectrum by X-ray absorption spectroscopy. The spectrum found in cisplatin-DNA samples is altered differently by the addition of either WR1065 or sodium azide. Importantly, a similar change in Pt edge spectra was noted in cells treated with cisplatin and WR1065. Therefore, amifostine should be reconsidered as a candidate for treatments that reduce or prevent CIPN.

Poster presentation

## THE NEUROPROTECTIVE EFFECTS OF TREHALOSE AGAINST 6-OHDA INDUCED OXIDATIVE STRESS IN HUMAN NEUROBLASTOMA SH-SY5Y CELLS

Danijela Stevanovic[1], Ljubica Vucicevic [2], Maja Misirkic Marjanovic[2], Verica Paunovic[1], Ljubica Harhaji-Trajkovic[2] and Vladimir Trajkovic[1]

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6-hydroxydopamine (6-OHDA) is one of the most common neurotoxins used to induce experimental model of Parkinson's disease (PD) both in vivo and in vitro. Neurotoxic action of 6-OHDA is mediated by oxidative stress and apoptotic cell death which leads to neurodegeneration and neuroinflammation. Natural disaccharide trehalose exhibits powerful neuroprotective effect in certain brain injury models. In this study, we investigated the neuroprotective and the antioxidative effects of trehalose against 6-OHDA induced neurotoxity in human neuroblastoma SH-SY5Y cells. The ability of trehalose to improve cell viability and to reduce neuronal death was assessed by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide), crystal violet, lactate dehydrogenase assay, AnnexinV-FITC/propidium iodide staining and cell cycle analysis. The production of reactive oxygen species (ROS) was analyzed by flow cytometry using redox-sensitive dyes dihydrorhodamine 123 (DHR) and MitoSOX Red. MTT and LDH assays demonstrated that trehalose pretreatment in a time-dependent manner significantly improved cell viability and reduced 6-OHDA induced neurotoxic effect. Annexin V-FITC and propidium iodide double staining and cell cycle analysis indicated that trehalose reduced 6-OHDA-induced apoptosis also in a time dependent manner. Flow cytometric analysis of DHR and MitoSOX stained cells confirmed that trehalose pretreatment significantly reduced oxidative stress and production of superoxide anion radical in 6-OHDA exposed cells. These results indicate that trehalose suppresses the formation of ROS and further protects from 6-OHDA induced neuronal death and suggest further investigation on trehalose as a potential treatment in oxidative neuronal injury such as Parkinson's disease.

Poster presentation

## GRAPHENE QUANTUM DOTS PROTECT SH-SY5Y CELLS FROM SNP INDUCED APOPTOSIS BY SCAVENGING REACTIVE OXYGEN AND NITROGEN SPECIES

Mihajlo Bosnjak[1], Biljana Ristic[2], Matija Krunic[2], Aleksandar Mircic[1], Nevena Zogovic[3], Gordana Tovilovic Kovacevic[3], Verica Paunovic[2], Vladimir Trajkovic[2], Ljubica Harhaji Trajkovic[3].

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We here investigated protective potential of nanoparticles graphene quantum dots (GQD) against neurotoxicity of sodium nitroprusside (SNP), NO-donor and antihypertensive drug widely used in studies of nitrosative stress-induced neurotoxicity. GQD prevented SNP-induced apoptosis, caspase activation and mitochondrial depolarization in SH-SY5Y neuroblastoma cells. GQD decreased SNP generated nitrite accumulation in supernatants, as well as NO/ONOO- concentrations in cells and cell-free medium. However, ONOO- and NO scavengers only slightly suppressed SNP neurotoxicity. Moreover, light exhausted SNP, incapable of producing NO, was toxic to SH-SY5Y cells, while GQD strongly reduced its neurotoxicity, suggesting that defensive effect of GQD far exceeded their NO scavenging activity. $FeSO_4$ increased death of SH-SY5Y cells, while iron chelators decreased toxicity of iron-containing SNP. GQD neutralized SNP generated reactive oxygen species (ROS) production, particularly $O_2$•− and •OH in both cells and cell-free condition. Neurotoxicity of SNP was suppressed in the presence of unspecific antioxidants, scavengers of •OH and lipid hydroperoxyl radicals, while it was increased with •OH generating superoxide dismutase (SOD). Intracellular localization of GQD was confirmed by transmission electron microscopy (TEM), while extensive washing of cells preincubated with GQD, only partly reduced their protective activity, suggesting that GQD exerted neuroprotective effect both intra- and extracellularly. Taken together, these results suggested that GQD protected neuroblastoma cells by neutralizing reactive nitrogen species (RNS) and ROS, predominantly •OH formed in Fenton reaction catalyzed by iron derived from SNP. Therefore, GQD might be promising choice for treatment of ROS/RNS-mediated neurodegenerative diseases.

Poster presentation

# ANTIOXIDANT DEFENSE ENZYMES ACTIVITY IN THE LIVER OF DIABETIC TYPE I MICE TREATED WITH METHANOLIC EXTRACT OF *Origanum vulgare*

Zorana Oreščanin Dušić[1*], Milica Vujčić[2], Nikola Tatalović[1], Teodora Vidonja Uzelac[1], Tamara Saksida[2], Aleksandra Nikolić Kokić[1], Mihajlo Spasić[1], Ivana Stojanović[2], Duško Blagojević[1]

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Our previous results showed that the administration of methanolic oregano extract (MOE) to C57BL/6 mice treated with multiple low doses of streptozotocin (MLSD) for diabetes induction, reduced diabetes incidence and preserved normal insulin secretion. MOE acts as an antioxidant and immunomodulatory and in an anti-apoptotic manner resulting in the protection of mice from diabetes development. Here we studied MOE effects on antioxidant defense enzymes activity (superoxide dismutase (SOD), catalsase (CAT), glutatione peroxidase (GPx) and glutathione reuctase (GR)) in the liver of 8-12 weeks old male C57BL/6 mice treated with MLDS (40 mg/kg per day, intraperitoneally for five consecutive days; Sigma-Aldrich). MOE was administered for ten consecutive days by intraperitoneal injections at a dose of 5 mg/kg per day, starting from the day of MLDS induction ('prophylactic' regimen). MOE was also administered to healthy mice for 10 days, and antioxidant enzymes were evaluated 14 days from the beginning of the experiment. The control mice received the vehicle (PBS + dimethyl sulfoxide (DMSO)). Results showed that MLDS treatment induced total SOD activity elevation and a decrease of GPx activity suggesting the state of oxidative stress. MOE abolished MLDS effect on SOD activity, but was ineffective concerning GPx activity. Since MOE has potent *in vitro* antioxidant activity measured by DPPH scavenge test, it seems that its protective effect comes from radical scavenging activity. However, there is more than one compound responsible for the beneficial effect of MOE. Therefore, its protective action has to be considered as a multitasking on both cellular and tissue level.

Poster presentation

## POLYMORPHISMS IN GLUTATHIONE S-TRANSFERASE T AND M GENES MAY SERVE AS DETERMINANTS OF RISK IN APICAL PERIODONTITIS

Aleksandar Jakovljevic[1], Nadja Nikolic[2], Jelena Carkic[2], Miroslav Andric[3], Maja Miletic[1], Katarina Beljic-Ivanovic[4], Jelena Milasin[2]

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Apical periodontitis (AP) represents an inflammatory process in periapical tissues characterized, among other, by the presence of oxidative stress. Glutathione S-transferases are important antioxidant enzymes involved in the detoxification of endogenous or exogenous compounds such as reactive oxygen species (ROS). Deletion polymorphisms in glutathione S-transferase genes, leading to decreased enzymatic activity, have not been previously associated with apical periodontitis. Therefore, this study aimed to investigate glutathione S-transferase theta 1 and mu 1 (GSTT1 and GSTM1) polymorphisms as determinants of risk in apical periodontitis. One hundred and twenty AP lesions were collected following standard apicoectomy. Control group consisted of 60 healthy pulp tissues harvested after third molar extraction. Genotyping for GSTM1 and GSTT1 deletion polymorphisms was performed by real-time PCR and melting curve analysis. Allele and genotypic frequencies, Hardy–Weinberg equilibrium (HWE) were calculated using the SPSS statistical software. Logistic regression analysis was used to calculate odds ratio (OR) and 95% confidence interval (CI). There was no evidence of deviation from HWE for any of the investigated polymorphisms within the groups ($p>0.05$). GSTM1 was deleted in 89 periapical lesions (74.2%) and in 31 controls (51.7%). The presence of GSTM1 null genotype led to an over 2.5-fold risk increase for the development of chronic apical periodontitis (OR = 2.69, 95% CI = 1.40-5.15, P = 0.003). GSTT1 gene was deleted in 72 periapical lesions (60%) and in only 2 controls (3.3%). GSTT1 null genotype significantly affected the risk for the development of apical periodontitis ($P < 0.001$), leading to an over 40-fold increase (OR = 43.5, 95% CI = 10.14-186.59). Beside other factors, heredity may contribute to variable predisposition to AP pathogenesis. The present study has demonstrated that deletion polymorphisms in *GSTT* and *GSTM* increase the susceptibility for development of apical periodontitis.

Poster presentation

## THE ROLE OF PKC-ζ AND IFNα IN REGULATION OF CELL: CELL CONTACT AND HEPATITIS C VIRUS (HCV) ENTRY INTO HEPATOCYTES

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The HCV is a major cause of chronic liver cirrhosis and hepatocellular carcinoma. While acute infection is usually asymptomatic and can be spontaneously cleared, approximately 50-80% of patients progress to chronic infection. The WHO estimates that 71 million people worldwide have chronic infection. Although, DAA (direct-acting antiviral) therapy is effective in around 95% of patients, universal access to diagnosis and treatment is very limited. Dissecting the nature of the immune response during HCV infection will aid vaccine development. HCV utilises different host factors to facilitate its cell entry, replication and propagation. Atypical PKCs (Protein Kinase C) direct the establishment of epithelia-specific junctional structures. These structures are composed of proteins such as claudin-1 and occludin, which are host factors required for HCV cell entry. Interferons are central to the regulation of the antiviral immune response with IFNα being the most abundant IFN released by numerous cell types following viral infection. In this study we have demonstrated that the atypical PKC isoform, PKC-ζ, is localised within the plasma membrane and at the cell:cell junctions of the Huh7 hepatocarcinoma cell line. PKC-ζ colocalises with adherens-junction and tight-junction proteins including claudin-1 and occludin. Inhibition of PKC-ζ activity, using a pseudosupstrate inhibitor, induced the relocation of PKC-ζ, claudin-1 and occludin to the cytosol and the disruption of cell:cell contacts. Furthermore, inhibition of PKC-ζ activity reduced the ability of HCV to infect Huh7 cells as demonstrated using a HCV *in vitro* cell culture system (HCVcc). We also demonstrated that IFNα induced the redistribution of PKC-ζ and directed a dynamic remodelling of cell:cell contacts through the translocation of PKC-ζ from the plasma membrane domain to the cytosol. Our findings reveal that PKC-ζ is a vital host cell factor required by HCV to enter and infect hepatocytes and identifies a novel antiviral mechanism employed by IFNα.

Poster presentation

## SEX-SPECIFIC EFFECTS OF TREATMENT WITH URB597, A SELECTIVE INHIBITOR OF FATTY ACID AMIDE HYDROLASE, ON IL-10 AND SPLEEN HYPERTROPHY OF RATS SUBJECTED TO THE CHRONIC UNPREDICTABLE STRESS

Harisa Ferizović[1], Milica Janković[1], Nataša Spasojević[1], Bojana Stefanović[1], Slađana Dronjak[1]

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There is growing evidence supporting the hypothesis that immune hyperactivation and dysregulated cytokine production are involved in depression. Overexpression of inflammatory cytokines is associated with bad prognosis in major depressive disorder, whilst many recent studies show positive modulatory role of anti-inflammatory cytokines, such as IL-10 in psychiatric disorders. Moreover, converging experimental data suggest important role of endocannabinoids in depressive disorders. Aim of this study was to examine the effects of URB597. Male and female Wistar rats were exposed to the 6 weeks of chronic unpredictable stress (CUS) and treated intraperitoneally with either 0.3 mg/kg/day of URB597 or vehicle in the last 2 weeks of stress protocol. IL-6 plasma levels and IL-10 levels in spleen were examined using Elabscience ELISA kits. Animals of both genders which were subjected to the CUS developed significant spleen hypertrophy. URB597 prevented spleen hypertrophy in male, but not in female animals. Both male and female rats showed elevated plasma levels of IL-6 caused by CUS which were not affected by URB 597 treatment. In contrast, animals subjected to the CUS showed lower levels of IL-10 in spleen compared to the control animals. URB597 increased levels of IL-10 in males, but not in females. Our results suggest that URB597 may have potentially beneficial anti-inflammatory effect in depression-induced inflammation through regulation of IL-10. However, this effect is sex-dependent and present only in males.

Poster presentation

## INFLUENCE OF AGEING ON SEX AND STRAIN DIFFERENCES IN IMMUNE RESPONSE TO INACTIVATED INFLUENZA VACCINE

Raisa Petrović[1], Nevena Arsenović-Ranin[2], Biljana Bufan[2], Irena Živković[1], Ivana Prijić[1], Vera Stoiljković[1], Gordana Leposavić[3]

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Efficacy of the immune response to vaccine depends on genetic background, sex and age of the recipient. However, mechanisms underlying this phenomenon have not been elucidated, yet. The study investigated influence of sex and age on serum IgG response to seasonal trivalent inactivated split influenza vaccine (TIV) in BALB/c and C57BL/6 mice, and mechanisms underlying this response. Total serum IgG responses to influenza virus type A strains declined with aging, in a strain-specific manner. Consequently, strain differences (greater IgG responses in BALB/c mice) observed in young mice (three-month-old) were abrogated in old (eighteen-month-old) ones. However, irrespective of strain and age, females developed stronger influenza type A-specific IgG responses than males. Despite age-related decrease in influenza B-specific serum IgG response, it was comparable between old BALB/c and C57BL/6 mice. The strain/sex-specific differences in age-related changes in the magnitudes of IgG responses to TIV correlated with those in number of germinal centre (GC) B splenocytes. These differences were related to those in B splenocyte and CD4+ splenocyte proliferation in culture upon restimulation with influenza viruses from TIV. The magnitudes of IgG responses also correlated to T follicular regulatory (Tfr)/T follicular helper and Tfr/GC B splenocyte ratios across all groups of mice. Aging, irrespective of influenza virus-specificity, affected serum IgG2a(c)/IgG1 ratios (reflecting IFN-γ/IL-4 production level ratio) in male BALB/c and female C57BL/6 mice, respectively. Thus, although in young mice of both strains these ratios were comparable between sexes, in old females they were shifted towards IgG1 when compared with age-matched males. Consistently, the IFN-γ/IL-4 production level ratios in splenocyte cultures stimulated with influenza viruses from old females of both strains were shifted towards IL-4 compared with that in age-matched male cultures. The study stimulates further research to formulate sex-specific strategies to improve efficacy of influenza vaccine in elderly. (Funding: project 175050, MNTR RS)

Poster presentation

## MODULATION OF FUNCTIONAL ACTIVITY OF GRANULOCYTES THROUGH PERIODONTAL LIGAMENT MESENCHYMAL STEM CELLS PRIMED WITH PROINFLAMMATORY FACTORS

Tamara Kukolj[1], Ivana Okić Đorđević[1], Mila Pešić[1], Slavko Mojsilović[1], Diana Bugarski[1], Aleksandra Jauković[1]

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Immunomodulatory functions of periodontal ligament stem cells (PD-SCs) in the context of granulocytes (GRA) activity regulation have not been examined so far. Therefore, the aim of this study was to investigate how proinflammatory factors, significant for periodontal disease progression, modulate interactions between PD-SCs and GRA.

As bacterial agents strongly activate innate immunity at the beginning of inflammatory reaction, first we analyzed potential ability of PD-SCs treated with lipopolysaccharide (LPS, *E. Coli*) to attract GRA through endothelial barrier. After 2h of incubation, by using transwell co-culture system, PD-SCs significantly reduced transendothelial migration (TEM) of GRA *in vitro*, while 72h pre-treatment with LPS (1000 ng/ml) did not affect this property of PD-SCs. Along with TEM, we examined the effect of PD-SCs and their conditioned medium (CM) on GRA respiratory burst *in vitro* based on NBT reduction test. We showed that PD-SCs have potential to inhibit respiratory burst of GRA (both stimulated and unstimulated) after direct co-culture, while the effect of proinflammatory factors varied. Namely, no changes of PD-SCs action were detected after LPS (1000 ng/ml) and IL-17 (50 or 100 ng/ml) treatment, while TNF-α (1, 10 or 20 ng/ml) amplified inhibitory functions of PD-SCs. Unlike direct co-culture tests, results related to the effects of CM on GRA (both stimulated and unstimulated) respiratory burst point out the inhibitory action of CM of PD-SCs pre-treated with TNF-α (10 or 20 ng/ml). On the other hand, CM derived from control PD-SCs and PD-SCs treated with IL-17 or LPS did not change GRA activity.

Considering the differences observed in direct co-culture and CM effects, these results indicate the existence of complex mechanisms in PD-SCs/GRA crosstalk. Moreover, the importance of the soluble factors present in microenvironment should be highlighted regarding their contribution in shaping the functional activity of PD-SCs as local regulators in inflammatory process.

Poster presentation

## TITTER AND AVIDITY OF SERUM ANTIBODIES REACTIVE WITH *Lactobacillus* spp. IS CHANGED IN LOCAL AND SYSTEMIC INFLAMMATORY DISEASES OF BONE TISSUES.

Dragana Smiljanić[1], Rajna Minić[2], Dragana Marković[3], Ivana Drvenica[3], Marijana Kovačić[3], Ana Stančić[3], Irina Maslovarić[3], Gavrilo Brajović[1], Miloš Hadži-Mihajlović[1], Mirjana Šefik-Bukulica[4], Vesna Ilić[3]

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The gut microbiota is necessary to establish an effective immune response, but also related to the onset of local and systemic inflammatory diseases of bonne tissue. Genus Lactobacillus is one of the main bacterial inhabitants of digestive tract, and we analysed, with in-house ELISA, titter and avidity of serum IgM, IgG, and IgA antibodies reactive with whole *Lactobacillus plantarum* and *Lactobacillus reuteri*, of 25 periodontal disease (PD) patients without any systematic disease, 26 rheumatoid arthritis (RA) patients, and of 21 healthy people. In healthy people we found no correlation between titters of different immunoglobulin isotypes reactive with Lactobacillus spp. Moderate correlation between IgG and IgA titters was detected in PD and RA. Only in RA, the correlation between IgG and IgM, and IgA and IgM titters was found. No difference between the groups in titters and avidity of anti-Lactobacillus IgG and IgA was found. However, both titter and avidity of IgM antibodies reactive with any of two Lactobacillus species was lower in RA then in PD and in healthy people. We also observed that serum antibodies "discriminated" two Lactobacillus species. Thus, in healthy people and in PD patients titter and avidity of IgG to L. *reuteri* were of significantly lower values than those to *L. plantarum.* Besides, high correlation between titters of IgG antibodies against *L. plantarum* and *L. reuteri* was found in all groups, high correlation between titters of IgA antibodies to two Lactobacillus sp. was detected in PD and RA, and of IgM antibodies only in RA. The results showed that the serum anti-Lactobacillus antibodies reactivity in PD and RA patients differed of the reactivity observed in healthy people. This might reflect a disturbed immunoglobulin reactivity caused by disease-related hyper stimulation and/or a disorder in the gut microbiome level. The significance of the results remains to be elucidated.

Poster presentation

# PHOTON CORRELATION SPECTROSCOPY ANALYSIS OF CIRCULATING IMMUNE COMPLEXES SIZE IN BRONCHOPNEUMONIC CALVES

Ivana Drvenica[1], Marijana Kovačić[1], Natalija Fratrić[2], Vesna Ilić[1]

[1]*Institute for Medical Research, University of Belgrade, Serbia*
[2]*Faculty of Veterinary Medicine, University of Belgrade, Serbia*

Immune response to certain pathogens in calf bronchopneumonia is mediated by IgG antibody and immune complexes which they form. Whether these immune complexes will be effective in eliminating the pathogen or will initiate pathological response, depends on their size. In this study we investigated the size of circulating immune complexes (CIC) in three-month old calves with bronchopneumonia caused by *Pasteurella multocida*, using photon correlation spectroscopy (PCS). PCS is a method used for a detection of aggregates in therapeutic antibodies preparations, but also for detection and characterisation of CIC in humans and experimental animals. Heat aggregated IgG represent an *in vitro* analogue of CIC, and we first analysed if the heat aggregated calf serum IgG can be detected by PCS. Before the heating, in calf serum protein fraction enriched with IgG we observed only particles of 10 nm in diameter (diameter of native bovine IgG). After the heating, only large aggregates having diameter of 532 nm were detected. The result confirmed that calf CIC can be estimated by means of PCS. Using PCS, in a pooled, PEG-precipitated, CIC sample of healthy calves, six types of particles different in diameter were found, and among them particles with diameters of 220 nm and 615 nm were dominant. In a pooled CIC sample of diseased calves four types of particles were identified. Particles with diameters of 165 nm and 1281 nm were dominant, and were approximately two (615 nm vs 1281 nm) and six times (220 nm vs 1281 nm) larger than the leading CIC particles of healthy calves. The measurement of electrokinetic potential confirmed that CIC of diseased calves were more stable than CIC of healthy calves. Whether the revealed increase in size and stability of CIC in calves with bacterial bronchopneumonia is of significance for their functionality remains to be analysed.

Plenary lecture

## DECREASE IN BLOOD GLUCOSE LEVELS DUE TO VIRAL INFECTION PROMOTES INNATE-IMMUNE ANTI-VIRAL RESPONSE

Marko Šestan[1], Ante Benić[1], Felix M Wensveen[1] and Bojan Polić[1]

[1]*Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia*

Viral infection has a major impact on systemic metabolism. We have recently shown that viral infection impacts endocrine regulation of systemic blood glucose levels (Šestan et al., Immunity 2018). However, it is not known how strength of infection reflects on blood sugar levels during infection, and if these changes are beneficial to the host. Here we investigated how different viral loads impact regulation of blood glucose levels. We showed that infection of mice with high, but non-lethal titers of mCMV or LCMV causes transient relative hypoglycemia. Low blood glucose levels were beneficial to the host as enforced hyperglycemia during infection resulted in significant increase in viral titers in peripheral organs. With LCMV, relative hypoglycemia was the result of IFNγ secretion by γδT cells, as δ deficient mice, and mice treated with anti-IFNγ antibodies did not develop this condition. Using mice without expression of IFNγ receptor on myocytes, we showed that IFNγ causes insulin resistance in muscle, which causes compensatory hyperinsulinemia. This, in turn, impairs glycogen utilization as a source of blood glucose, resulting in low blood glucose levels. *In vitro*, we could show that low glucose concentrations in medium causes an increase in cellular stress, which made cells less receptive for viral replication and reduced viral titers. In summary, we found that infection with high, non-lethal titers of virus causes relative hypoglycemia. This causes systemic cellular stress, which makes the organism less receptive for viral replication. Thus, we speculate that reduced blood sugar levels during infection are part of body's natural response to infection.

Saturday, December 7th

Plenary lecture

## APPLIED OMICS: INSIGHTS INTO THE PATHOGENESIS OF MULTIPLE SCLEROSIS

Maja Jagodić

*Department of Clinical Neuroscience, Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden*

Multiple Sclerosis (MS) is a chronic inflammatory disease characterized by autoimmune destruction of myelin and neurons in the central nervous system. Today, MS is one of the leading causes of neurological disability in young adults. Current treatments act broadly on the immune system and they are effective in controlling the inflammatory stage of disease while there are no treatments that prevent sustained neuronal loss and disease progression. Although the cause of MS remains unknown, vast epidemiological data establish MS as a complex disease influenced by genetic and environmental factors. Epigenetic mechanisms, such as DNA methylation, orchestrate activity of the genome in response to environmental cues and may provide better understanding of disease pathogenesis. One of the main challenges with studying diseases such as MS is the limited access to the target tissue - *the brain*. Recent progress in development of methods to survey epigenetic modifications, and from them infer genome activity, opened up possibilities to study brain tissue and mechanisms that underlie neuronal loss in MS patients.
To understand the mechanisms of disease development and progression, we are utilizing unique clinical cohorts in combination with state-of-the-art methods to measure DNA methylation and transcription in discrete cell types, followed by functional studies in experimental models *in-vitro* and *in-vivo*.

Saturday, December 7th Session: METAB INFLAMM

Invited lecture

## NUTRIGENOMICS AND INFLAMMATION

Rosita Gabbianelli, Cinzia Nasuti, Donatella Fedeli, Laura Bordoni

*Unit of Molecular Biology, School of Pharmacy, University of Camerino, 62032 Camerino (MC), Italy.*

Diet is one of the main factors that can impact gene expression and gut microbiota diversity and it provides the functional groups required for epigenome modulation.

Starting from early life, the exposome (e.g. diet, xenobiotics, drugs, etc.) is responsible for specific epigenetics marks. A maternal high fat diet and food pesticide exposure during the pre- and post-natal period of live can promote inflammatory responses in offspring that may influence organ development promoting a healthy/unhealthy phenotype in adulthood. High protein and carbohydrate (e.g. fructose, glucose) intake in adult age can play a key role in the development of chronic low-grade systemic inflammation. Of particular concern is that epigenetic marks of diseases may be inherited, hence they can mediate epigenetic inheritance of diseases.

In this context, nutrigenomics mediated by bioactive compounds and a balanced nutrient intake can actively control inflammation and maintain the cellular redox homeostasis.

A summary of data on how and when nutrigenomics can modulate inflammatory responses through healthy dietary choices to prevent the main inflammatory-related metabolic diseases occurring across life will be presented.

Saturday, December 7th Session: METAB INFLAMM

Short oral presentation

## THE IMMUNOMODULATORY EFFECTS OF POLYPHENOL RICH JUICE CONSUMPTION ON GENE EXPRESSION IN SUBJECTS AT CVD RISK

Aleksandra Stankovic[1], Ljiljana Stojkovic[1], Manja Zec[2]

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Anthocyanins (ANTs) belonging to the flavonoids subclass of polyphenols, related to the prevention of the development of chronic diseases. The chronic inflammation is common in many complex diseases: metabolic syndrome, diabetes and cardiovascular diseases. *In vitro and in vivo* experiments in animals showed modulating effects of ANTs on cardiovascular risk factors such as central obesity, dyslipidemia, hyperglycemia and hypertension, which occurs simultaneously in cardio-metabolic disorders. Aim of the study was to delineate the effects of polyphenol-rich *Aronia melanocarpa* juice, daily consumption, on the transcriptome of PBMC (which have been used as common target in nutrition studies to reflect changes in metabolic inflammation) in subjects at moderate CVD risk (defined as the presence of at least one of the following: increased BMI, central obesity, high normal blood pressure or dyslipidemia). The present research is designed as a sub-study of the original three-arm, crossover, randomized, double-blind, placebo-controlled study (ClinicalTrials.gov as NCT02800967). Three 4weeks treatments were: original Aronia juice (high dose of total polyphenol, 11771.09 mg gallic acid equivalents (GAE)/L); Aronia juice-based beverage (low dose of polyphenol, 2942.77 mg GAE/L); and placebo beverage. The microarray analysis was performed at two time points (before and after each treatment). Enrichment analysis of DEGs will be presented. The analysis revealed 10 Hallmark gene sets significantly enriched and upregulated after original Aronia juice consumption. Top 3 enriched gene sets were TNFA signaling via NFKB, apoptosis and inflammatory response, which is in line with findings of the current enrichment analysis of DEGs. The overlapping and non-overlapping significantly enriched biological terms among treatments were defined. In conclusion, daily Aronia juice consumption significantly affected transcriptome in subjects at moderate CVD risk after both, short-term and long-term treatments. Validation analysis of differentially expressed genes is in progress.

Saturday, December 7th Session: METAB INFLAMM

Short oral presentation

## T CELLS AND STRESS - NOVEL REGULATORS OF OBESITY DEVELOPMENT

Ivana Nikolić, Luis Leiva-Vega, Alfonso Mora, Aránzazu Pintor Chocano, María Elena Rodríguez Andrés, Magdalena Leiva, María Crespo-Ruiz, Marta Pulgarín-Alfaro, Marta León, Aikaterini Tsilingiri, Pilar Martin, Guadalupe Sabio

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Obesity is currently a serious epidemic health problem associated with other metabolic disorders. The hallmark of obesity is inflammation, especially in adipose tissue (WAT), liver, pancreas and blood. Pro-obesogenic immune response is driven by pro-inflammatory M1 macrophages, cytokines, Th1 and Th17 cells and reduced response of anti-inflammatory Th2 and regulatory T cells (Treg), especially in WAT. In addition, obesity promotes senescence of memory T cells and depletion of $CD4^+$ cells protects against weight gain. p38 kinases (p38α, p38β, p38γ, p38δ), an important kinase family implicated in the transduction of stress signals into the cell, could be the key regulator of obesity development. Activated by kinases MKK3 and MKK6, p38s promote Th1/Th17 response and negatively regulate Treg cell induction. To clarify the role of p38s pathway in Treg and memory T cell development, and its role in obesity, we generated mice lacking MKK3/6 in $CD4^+$ cells ($MKK3/6^{CD4\text{-}KO}$). Our data showed that these mice have reduced number of $CD4^+$ T cells, upregulated Treg cells in blood and reduced frequency of memory T cells with aging. After feeding an HFD, $MKK3/6^{CD4\text{-}KO}$ mice had lower body weight and glucose level in blood. Furthermore, mice lacking MKK3/6 in $CD4^+$ cells had higher energy expenditure, higher brown adipose tissue thermogenesis and less ectopic fat accumulation in liver. Our results suggest that mice lacking MKK3/6 in $CD4^+$ T cells are protected against diet-induced obesity and T2D by improving their whole body metabolism. Further study will help us to decipher the molecular mechanism responsible for observed protective phenotype. These findings will allow us to create novel, cell specific therapeutics approaches to efficiently fight obesity and its related diseases.

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Saturday, December 7th Session: METAB INFLAMM

Short oral presentation

## IS METAFLAMMATION A USUAL SUSPECT FOR FRUCTOSE-INDUCED METABOLIC DISTURBANCES?

Nataša Veličković, Danijela Vojnović Milutinović, Jelena Brkljačić, Ana Teofilović, Biljana Bursać, Marina Nikolić, Ljupka Gligorovska, Sanja Kovačević, Gordana Matić, Ana Djordjevic

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Fructose overconsumption, especially in the form of sweetened beverages, has been linked to development of obesity, insulin resistance, dyslipidemia and type 2 diabetes. In rodents, high-fructose diet leads to hypertriglyceridemia, ectopic fat deposition and insulin resistance. Metabolically triggered inflammation (metaflammation) is now recognized as a link between nutrient signals and insulin resistance and considered as usual suspect for metabolic disturbances. Metaflammation usually evolve from visceral adipose tissue, progresses to liver and brain strucures, and results in peripheral insulin resistance, lipid accumulation and oxidative stress. Hence, the aim of our study was to investigate metaflammation as a trigger for fructose-induced metabolic disturbances. Experiments were performed on male Wistar rats fed with different concentrations of liquid fructose (10, 20 and 60%) during 9 weeks. Physiological and biochemical parameters, hepatic and brain inflammation, indicators of peripheral and systemic insulin resistance, as well as hepatic lipogenesis and oxidative stress were examined. The results demonstrated that fructose-enriched diet generally led to increased proinflamatory cytokines in the liver, hippocampus and hypothalamus, and to stimulated activation of proinflamatory kinases NFκB and JNK, while it did not change the expression of inflammasome component NLRP3, toll-like receptor 4 or anti-inflammatory cytokines in the liver. The observed metabolic inflammation was accompanied with impaired glucose tolerance after 10 and 20% fructose-enriched diet, while decreased hepatic insulin sensitivity, hypetriglyceridemia and increased expression of hepatic lipogenic genes were observed after all fructose diets. The treatment of fructose-fed rats with chronic unpredictable stress annulled the effects of fructose on hepatic and hypothalamic inflammation and glucose tolerance, but did not alter fructose-induced effects on lipogenesis and insulin signaling. The results suggest that fructose-induced metaflammation and systemic insulin resistance are closely interconnected, while the link between inflammation and other metabolic disturbances could still be a matter of debate.

Poster presentation

## PROPHYLACTIC TREATMENT BY BANANA LECTIN INFLUENCES THE IMMUNE RESPONSE IN THE PEAK OF EXPERIMENTAL COLITIS

Radmila Miljković[1], Ivana Lukić[1], Emilija Marinković[1], Ana Kovačević[1], Mina Popović[2], Zorana Lopandić[3], Marija Gavrović-Jankulović[3], Marijana Stojanović[1]

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Banana is highly abundant in everyday nutrition and it is recommended to be a part of the diet with patients suffering from inflammatory bowel diseases. A mannose-specific banana lectin (BanLec) is reported to be a potent immunomodulator. Our aim was to explore the prophylactic impact of BanLec in a murine model of colitis. A recombinant BanLec (rBanLec) that possesses structural and functional characteristics similar to naturally occurring counterparts was used in the research. Colitis was induced (day 0) in C57BL/6 mice by 2,4,6-trinitrobenzene sulfonic acid (TNBS; 3.5 mg/ml TNBS / 50% ethanol, 100 μl intrarectal). 24h prior to the induction of colitis, mice were treated (100 μl, intrarectal) by 0.1 μg/ml (rBL0.1), 1 μg/ml (rBL1) and 10 μg/ml (rBL10) rBanLec/PBS. Mice subjected to colitis induction without rBanLec pretreatment (PC) were referent. The impact of rBanLec pretreatment was assessed at the peak of the disease (day 2). Body weight loss was taken as a main parameter for estimation of disease severity. A significant reduction in disease severity was noticed in rBL0.1 group and it correlated with lower leukocyte infiltrations in the colon. Nevertheless, inflammation-related parameters (MPO activity, production of NO, IL-12 and TNFα) were significantly lower in colons of rBL0.1 group compared to rBL1, rBL10 and PC groups. Levels of regulatory cytokines (IL-10, TGFβ) were the highest in the colons of rBL0.1 group. Local activities of antioxidant enzymes (CAT, SOD, GST) were also significantly increased in rBanLec-pretreated groups, especially in rBL0.1 group.

Presented results show that local stimulation by low dose of rBanLec prior to colitis induction reduces the severity of the disease. Observed positive impact resulted from the stimulation of local regulatory and antioxidant mechanisms that alleviate harmful proinflammatory response. (Supported by Ministry of Education, Science and Technological Development Republic of Serbia, Grant no. 172049)

Poster presentation

# INFLAMMATION AND INSULIN SENSITIVITY IN THE LIVER OF FRUCTOSE-FED *Mif* DEFICIENT MICE

Ljupka Gligorovska[1], Ana Teofilović[1], Nataša Veličković[1], Danijela Vojnović Milutinović[1], Sanja Kovačević[1], Gordana Matić[1] and Ana Djordjevic[1]

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Introduction: The macrophage migration inhibitory factor (MIF) is a key pro-inflammatory mediator involved in the regulation of energy metabolism and metabolic inflammation in the liver. Fructose overconsumption has been previously associated with development of low-grade inflammation characterized by elevated production of pro-inflammatory cytokines and activation of mitogen-activated protein kinase (MAPK) signaling pathway. The inflammatory response can disrupt insulin signaling and genetic deletion of *Mif* may contribute to the development of systemic insulin resistance, as well. The aim: The aim of the present study was to elucidate combined effects of *Mif* deficiency and fructose-enriched diet on metabolic inflammation and insulin sensitivity in the liver of male mice. Methods: Wild type (WT) and *Mif* deficient ($MIF^{-/-}$) C57Bl/6J mice were used to analyze the effects of 9-week 20% fructose-enriched diet on indicators of insulin sensitivity and markers of metabolic inflammation (tumor necrosis factor α (TNFα), interleukin (IL)-1β and IL-6). Deregulation of Akt signaling pathway was used as hallmark of hepatic insulin resistance. Also, the protein levels of extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase 1 (JNK) and p38 were analyzed. Results: *Mif* deficient animals exibited elevated expression of IL-1β and IL-6 in the liver, regardless of the diet regime, while hepatic TNFα was unchanged in all animals. On the other hand, both total and phosphorylated ERK and JNK protein levels were decreased in all fructose-fed mice. In the same animals, impaired hepatic insulin signaling, revealed by decreased pAkt and total Akt protein levels, was observed. Conclusion: Although, *Mif* deficiency led to upregulation of pro-inflammatory cytokines, fructose diet did not aggravate this effect. On the other hand, insulin signalling was diminished by fructose feding independently of *Mif* deficiency.

Saturday, December 7th Session: METAB INFLAMM

Poster presentation

## AN ANTI-DIABETIC DRUG, DIPEPTIDYL PEPTIDASE 4–INHIBITOR INDUCE TOLEROGENIC ILT4-EXPRESSING HUMAN DENDRITIC CELLS

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Dipeptidyl peptidase (DPP)-4 inhibitor belong to a class of anti-hyperglycemic agents indicated for patients with type-2 diabetes. In addition to glucose-lowering effects, emerging data suggests that DPP4 inhibitors could display anti-inflammatory effects as well, but the molecular and immunological mechanisms of these actions are insufficiently investigated. Using a model of human monocyte-derived dendritic cells (MoDC), which are the key immune regulatory cells we investigated how non-toxic concentrations of Sitagliptin (250 μg/ml) affect DC properties, morphology, differentiation and maturation potential, as well as the capacity to modulate T cell-mediated immune response *in vitro*. We found an inhibited DC differentiation, according to CD14/CD1a co-expression analysis, after the differentiation of MoDC in the presence of sitagliptin. These results correlated with a weak maturation capacity of sitagliptin-treated MoDC upon stimulation with LPS/IFN-γ and their lower capacity to stimulate T cell proliferation in co-culture, compared to control MoDC. Moreover, sitagliptin-treated MoDC displayed a higher expression of immunoglobulin like-transcript (ILT) 4 tolerogenic marker and produced significantly more TGF-β. In co-culture with T cells, sitagliptin-treated MoDC potentiated Th2 polarization and induced a higher percentage of $CD4^{+}CD25^{high}Foxp3^{+}TGF\text{-}\beta^{+}$ regulatory T cells, compared to corresponding control MoDC. Interestingly, $CD4^{+}CD25^{high}Foxp3^{+}$ Tregs induced by sitagliptin-MoDC also displayed a high expression of CD127, suggesting their memory Treg phenotype. Our results suggest for the first time that DPP4 inhibitor can induce tolerogenic properties in DCs, which could explain some of the anti-inflammatory actions observed for DPP-4 inhibitors. Besides glucose lowering effects, these tolerogenic properties DPP-4 inhibitors, could be beneficial for prevention of microvascular and macrovascular complications in diabetic patients, but also in the therapy of inflammatory diseases.

Saturday, December 7th Session: METAB INFLAMM

Poster presentation

## THE EFFECTS OF FISH OIL INTAKE ON SOME IMMUNOLOGICAL AND ANTIOXIDATIVE PARAMETERS IN DM2 PATIENTS

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High density lipoprotein (HDL)-cholesterol and has been linked to vascular disease and diabetes. HDL also has antioxidative, anti-inflammatory, and antithrombotic properties. HDL-associated PON1 is primarily responsible for the properties of HDL in retarding the oxidation of LDL. The objective of this study was to determine the effect of fish oil consumption on antioxidative capacity of HDL-cholesterol by measuring erythrocyte activity of antioxidant enzymes [catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and PON1], parameter of oxidative stress, also measuring fasting blood lipids, glucose, insulin, creatinine, high sensitivity C-reactive protein (hsCRP) and HbA1c.

Twenty patients (12 females, 8 males, mean age 53±6) received daily 4g natural fish oil (1.2g n-3 fatty acids; 20:5n-3 [EPA] and 22:6 n-3 [DHA]) during 12 weeks.

The blood samples were taken before and at the end of the twelve weeks n-3 PUFAs intervention period. The fasting blood lipids, glucose, insulin, creatinine, high sensitivity C-reactive protein (hsCRP) and HbA1c were assessed from the same blood samples with a conventional autoanalyzer. Blood samples were taken after 12h of overnight fasting. Serum CRP levels were measured using a highsensitivity CRP (hs-CRP) assay. The assay was able to detect a minimum hs-CRP concentration of 0.175 $mg \cdot L^{-1}$.

After supplementation with n-3 PUFAs HbA1c was improved, and not significant improve in glucose level, HOMA-IR and HOMA-beta. The level of HDL-C improved with borderline significance, by 19% ($p < 0.05$). There was no significant improvement of hsCRP level after intervention, although there was a trend to decreased 17.5% ($p=0.20$) compared to baseline levels. The level of SOD improved significantly, increased by 23.5% ($p=0.04$). We demonstrated significant improvement in PON1 activity ($p=0.01$), it was increased by ~ 105 %, as well as HDL-C corrected PON1 activity or PON/HDL-C ratio, increased by ~ 95 % ($p=0.01$).

## Saturday, December 7th Session: IMMUNODEF

### Invited lecture

### AUTOIMMUNITY AND AUTOINFLAMMATION IN PRIMARY IMMUNODEFICIENCY

Mario Abinun

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Besides conventional primary immunodeficiency disorders (PID) characterized by increased susceptibility to various infectious agents, the current classification of monogenic inborn errors of immunity (IEI) includes an increasing number of diseases of immune dysregulation characterized by autoimmunity, autoinflammation, allergy and malignancy (Picard C et al, 2018). Next generation sequencing techniques used for identifying mutations in genes involved in the innate and adaptive immunity played a major role in this progress. Both gain-of-function (GOF) and loss-of-function (LOF) mutations causing a number of novel monogenic autoimmune and autoinflammatory disorders have been recently reported (e.g. STAT1 and STAT3 GOF, CTLA4 haploinsufficiency, LRBA deficiency, PIK3CD and PIK3R1 GOF, otulin deficiency, RIPK1 deficiency, etc.).

As a number of the genes affected by GOF mutations also harbor LOF alleles, these 'experiments of nature' lead to a vast diversity of both the immunological and clinical phenotypes, mirroring the diversity and pleiotropy of the underlying genotypes. Clinical features are extremely variable, and can include systemic inflammation (fever and raised inflammatory markers), joint (arthralgia, inflammatory arthropathy) and skin involvement (vasculitis, vasculopathy, eczema, granulomas, blisters, panniculitis), interstitial lung disease, lymphadenopathy, hepato- and splenomegaly, inflammatory bowel disease (early onset severe enteropathy, colitis), organ specific autoimmunity (cytopenias, endocrinopathies), and presence of autoantibodies.

I will review the option of specific targeted therapies (such as JAK- and P110delta-signaling inhibition, etc.), developed in parallel to and as a result of better understanding of the underlying pathophysiology (Forbes LR et al, 2018), as well as our experience with allogeneic haematopoietic stem cell transplantation for the most severely affected patients, which are often resistant to these 'precision' therapies (Slatter MA et al, 2016; Nademi Z et al, 2017; Leiding JW et al, 2018; Greco R et al, 2019).

Saturday, December 7th Session: IMMUNODEF

Invited lecture

# PRIMARY IMMUNE DEFICIENCY AND CANDIDIASIS FROM BENCH TO BEDSIDE

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*Candida* is an opportunistic yeast causing infection in permissive circumstances, either secondary to recognized causes (e.g. tissue maceration) or due to immune gene mutations leading to Primary Immune Deficiencies (PIDs). Chronic Mucocutaneous Candidiasis (CMC) is persistent *Candida* infection of skin and mucous membranes, due to impairment of the STAT3/interleukin-17 pathway as a consequence of primary genetic defects of the immune system.

CMC may present 1) as part of broader immune defects (e.g. SCID), when it is accompanied by an array of other infections; 2) as part of defined syndromes (e.g. Hyper IgE - HIES); or as isolated disease (CMC disease - CMCD, see below). In 2011, in CMCD patients, we (N Engl J Med) and others (J Exp Med) identified a STAT1 gain-of-function (GOF) genetic mutation leading to downregulation of the STAT3/interleukin 17 pathway, found in >50% of all CMCD patients. The other major group of CMCD patients present with the Autoimmune Polyendocriopathy Syndrome type 1 (APS1) due to a genetic defect of the Autoimmune Regulator (AIRE) gene resulting in defective thymic clearance of autoimmune T cells, where we and others identified (JEM 2011) autoantibodies to IL-17 leading to CMC.

In a recent collaborative review of 276 patients (Blood 2016) we reported that GOF-STAT1 CMCD is a life-limiting condition with serious infectious, autoimmune and malignant complications. Diagnosis of GOF-STAT1 CMCD requires confirmation of pathogenic STAT1 mutations resulting in STAT1 hyperactivity (hyparphosphorylation & increased gene transcription). In APS1, anti-IL-17 antibodies have sensitivity and specificity of >98% mostly eliminating the need for complicated genetic confirmation. Unfortunately, these tests are still mostly available only in research laboratories. Defining the pathogenesis of GOF-STAT1 and APS1 CMCD has enabled new, bold therapeutic approaches including JAK-STAT inhibitors, anti-cytokine antibodies and bone marrow transplantation. PIDs are paramount in elucidating immune system function in both health and disease.

Saturday, December 7th Session: IMMUNODEF

Invited lecture

# PRIMARY IMMUNODEFICIENCY DISEASES WITH DEFICIENT ANTIBODY PRODUCTION IN CHILDREN

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Primary immunodeficiency diseases (PID) represent a group of inherited disorders of immune system with unusual susceptibility to various infections. Among PID, disorders of deficient antibody production are the most common.

In 1950, Ogden Bruton recognized X-linked agammaglobulinemia (XLA) in a 9-year-old boy with recurrent pneumococcal infectons. Later on, it was found that XLA is due to mutations of Bruton tyrosin kinase (btk), an enzyme which is important for development of mature B-cells in bone marrow. The children with XLA suffer from repeated respiratory infections with incapsulated bacteria. Repeated infections lead to bronchiectasis and chronic lung diseases. Intravenous immunoglobulin treatment, which was introduced in 80', is highly effective in preventing recurrent bacterial infections in these patients.

Common variable immunodeficiency (CVID) represents a group of heterogeneous immune disorders characterized by defect in antibody production. An incidence range from 25,000-50,000. It may develop as early as 2 years of age, with peak incidence in the third decade of life. In contrast to XLA, CVID is frequently associated with autoimmune disorders such as autoimmune cytopenias, autoimmune hepatitis, thyreoditis, rheumatoid arthritis, etc. Also, patients with CVID may develop malignant lymphomas. These patient also suffer from recurrent infections, and regular IVIG supstitution is necessary in prevention.

Selective IgA deficiency (sIgAD) is the most common PID in humans. It occurs with a frequency of 1:700-18,000 in different populations. Patients sIgAD may be healthy or present with recurrent infections. Also, allergic and autoimmune diseases can be associated with sIgAD. If associated with subclass IgG deficiency, development of bronchiectasis is consequence of repeated infections. These patients require IVIG replacement therapy.

In conclusion, PID with deficit of antibody production are common. IVIG replacement therapy is of vital importance preventing infections and chronic lung disease. Also, treatment with antibiotics is important in acute infections. In CVID, treatment of autoimmune phenomena requires the use of immunosuppressive drugs.

Saturday, December 7th Session: IMMUNODEF

Invited lecture

## THE MANY FACES OF HEREDITARY ANGIOEDEMA

Slađana Andrejević

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Patients with hereditary angioedema (HAE) have episodic swellings that can affect any part of the body. Most episodes result in reversible disability lasting 1–5 days. Studies in the early 1960s established that the fundamental defect in HAE was a deficiency of C1 inhibitor (C1INH). In the case of type I HAE, C1INH protein levels are low, whereas type II HAE is associated with reduced C1INH function due to a dysfunctional protein. Subsequent studies proved that type I and type II HAE resulted from mutations in the gene encoding C1INH, SERPING1. Until now more than 450 different mutations of SERPING1 gene have been reported. In 2000, two groups independently described families with HAE in which the affected subjects had entirely normal C1INH levels and function. When initially described, HAE with normal C1INH levels was called type III HAE, but it has become increasingly clear that type III does not represent a single type of HAE. In 2006 it was discovered that some families with HAE with normal C1INH levels had mutations in exon 9 of the coagulation factor XII (F12) gene that were shown to cause enhanced susceptibility of the contact system to become activated. Yet, only 20% of patients with HAE with normal C1INH levels in Europe have a F12 mutation. In 2018 a novel mutation in the angiopoietin 1 gene (ANGPT1) was identified in an Italian family with HAE. Another group has recently discovered a mutation in the plasminogen gene identifying a fourth type of HAE with normal C1INH levels. The most recent finding is kininogen 1 gene mutation that change N-terminal site of bradykinin. Our understanding of the basic biology of angioedema has dramatically expanded over recent years. Moreover, new insights into the pathophysiology of angioedema enabled therapies that changed the life of patients with HAE.

Saturday, December 7th Session: IMMUNODEF

Short oral presentation

# COMMON VARIABLE IMMUNODEFICIENCY: 18-YEAR FOLLOW-UP OF PATIENTS IN CLINICAL CENTER OF SERBIA

Radovan Mijanovic[1.2]

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[2]*Faculty of Medicine, University of Belgrade, Belgrade, Serbia*

Introduction: Common variable immunodeficiency (CVID) is heterogeneous syndrome characterized by hypogammaglobulinemia, defective antibody production, recurrent bacterial infections and presence of various immune disorders.
Material and Methods: The demographics, immunologic parameters, medical complications, pulmonary function tests and mortality statistics from 51 patients with CVID followed during 18 years (1998-2016; median 11±10,03) in Clinic of allergy and immunology, Clinical Center of Serbia, Belgrade, Serbia were analyzed in this study.
Results: The average age at diagnosis of CVID patients was 33 (10-68) years and the median delay in diagnosis was 6 (0-31) years. At the time of diagnosis median immunoglobulin (Ig) G level was 2.09, IgA: 0.2 and IgM 0.22 g/L. The commonest non-infectious complications were chronic lung disease in 52.9%, splenomegaly in 47.0%, bronchiectasis in 35.3%, hepatomegaly in31.4%, lymphadenopathy in 31.4% patients. Pernicious anemia was diagnosed in 16%, cytopenias in 13.7%, enteropathy in 11.8%, granuloma in 9.8% and lymphoma in 5.9% patients. The most frequent clinical manifestations prior to diagnosis of CVID were pneumonias in 85.1%, sinusitis in 68.6% and bronchitis in 47.1% patients. Infections were most often caused by *S. pneumoniae*, *H. influenzae*, *Pseudomonas a.* and *Giardia lamblia*. Males and females were affected equally by CVID, but in our group females were found to be older (53.5 vs. 41 years; p= 0.034) than males. 5-years survival rate in CVID patients was 91.4%. Mortality of this group of patients during 18 years follow-up was 25.5%. The most common causes of death were sepsis and respiratory failure. Reduced survival was associated with the delay in the diagnosis, initial high serum IgM levels and damaged lung function.
Conclusion: This is the first study of adult patients with CVID in Serbia that determined the spectrum of clinical manifestations, demographic and immunological characteristics, prognostic markers and disease outcome.

Saturday, December 7th Session: IMMUNODEF

Poster presentation

# THE ASSOCIATION BETWEEN SNP RS3212227 IN *IL12B* GENE AND COMMON VARIABLE IMMUNODEFICIENCY IN SERBIAN POPULATION

Vladimir Perovic[1], Dijana Perovic[2], Dusan Popadic[1], R. Mijanovic[3], B. Bonaci-Nikolic[3]

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Introduction: Common variable immunodeficiency disease (CVID) is a primary immunodeficiency characterized by impaired production of antibodies and increased susceptibility to infections. The variety in clinical presentation and genetic heterogeneity among patients with CVID represents considerable diagnostic challenge. The increasing body of evidence showed that genetic variants of cytokine genes may be involved in dysfunctional immune response in CVID. Genes *IL12A* and *IL12B* are encoding proinflammatory cytokine IL-12 which regulate cellular and humoral immunity. Considering important role of IL-12 in immune response, we investigated potential association between single nucleotide polymorphism (SNP) in *IL12B* +1188A>C (rs3212227) and CVID pathogenesis.

Materials and Methods: This study included 35 CVID patients and 250 healthy controls. Genotyping for *IL12B* +1188A>C was performed using TaqMan® genotyping SNP assays.

Results: Analysis revealed significant difference in allele and genotype distribution of polymorphism rs3212227 between controls and patients with CVID. In patients there was increased frequency of C allele (p=0,002) with odds ratio (OR) 2.32 and 95% confidence interval (95%CI) 1.35-3.99. Also, the frequency of CA genotype was significantly increased in patients (p=0.01; OR=2.50 95%CI=1.22-5.11), while AA genotype was more frequent in controls (p=0.02; OR=0.33, 95%CI=0.16-0.68).

Conclusions: This study indicates that polymorphisms in gene coding *IL12B* could have role in CVID susceptibility but our finding has to be confirmed in larger cohorts.

Saturday, December 7th Session: NEUROIMMUNO

Invited lecture

## INFLAMMATORY NEURODEGENERATION IN MULTIPLE SCLEROSIS

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Neurons in the central nervous system are organized in functional units interconnected to form complex networks. Acute and chronic brain damage disrupts brain connectivity producing neurological signs and/or symptoms. In several neurological diseases, particularly in Multiple Sclerosis (MS), structural imaging studies cannot always demonstrate a clear association between lesion site and clinical disability, originating the "clinico-radiological paradox." The discrepancy between structural damage and disability can be explained by a complex network perspective. Both brain networks architecture and synaptic plasticity may play important roles in modulating brain networks efficiency after brain damage. In particular, long-term potentiation (LTP) may occur in surviving neurons to compensate network disconnection. In MS, inflammatory cytokines dramatically interfere with synaptic transmission and plasticity. Importantly, in addition to acute and chronic structural damage, inflammation could contribute to reduce brain networks efficiency in MS leading to worse clinical recovery after a relapse and worse disease progression. This evidence suggests that removing inflammation should represent the main therapeutic target in MS. Moreover, as synaptic plasticity is particularly altered by inflammation, specific strategies aimed at promoting LTP mechanisms could be effective for enhancing clinical recovery. Better knowledge of features inducing neuronal degeneration and brain disconnection in MS is crucial to design specific strategies to promote recovery with increasingly tailored approach.

Saturday, December 7th Session: NEUROIMMUNO

Invited lecture

## NEUROFILAMENT LIGHT CHAIN LEVELS AND OLIGOCLONAL IgG BANDS - POTENTIAL BIOMARKERS IN MULTIPLE SCLEROSIS

Jelena Drulović

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Multiple sclerosis (MS) is a disabling disorder of the central nervous system characterised by immune-mediated demyelination and neurodegeneration. Significant effort has been made during the last decades to identify biomarkers for MS that may improve disease diagnosis and predict disease progression and activity. Neurofilaments (Nf) are structural elements of neurons, composed of three Nf chains (light, medium and heavy), which are released in the extracellular space following neuronal death. Therefore, Nf are considered a candidate biomarker of ongoing neurodegeneration. It has been demonstrated that serum Nf light chain correlates with concurrent and future clinical and MRI measures of disease activity and severity. High serum neurofilament light chain levels are associated with both brain and spinal cord volume loss. Cerebrospinal fluid (CSF) IgG oligoclonal bands are one of the crucial findings for establishing the diagnosis of MS, according to the new 2017 criteria. Additionally, they are known to play prognostic roles in patients with clinically isolated syndrome (CIS), and patients with positive oligoclonal bands are at higher risk for MS independently of other covariates present at the time of the CIS event. Also, in patients with radiologically isolated syndrome (RIS), oligoclonal bands have been demonstrated to be independent predictors of later conversion to CIS and MS. Similar to high CSF Nf light chain levels, the presence of oligoclonal bands was shown to be associated with shorter time to CIS and MS compared to individuals without oligoclonal bands. Evidence that serum and CSF Nf light chain levels and oligoclonal IgG bands may serve as diagnostic, prognostic and monitoring biomarkers in MS is progressively increasing.

Saturday, December 7th Session: NEUROIMMUNO
Invited lecture
THE GUT-BRAIN AXIS IN CNS AUTOIMMUNITY

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Autoimmunity results from a combined influence of genetic and environmental factors. Emerging evidence in the experimental models of autoimmunity suggests an important contribution of gut microbiota in the disease development. However, how and which gut microbial species are involved in triggering processes is poorly understood. In this presentation, I will present evidence for the role of gut microbiota in mouse models of Multiple Sclerosis, an autoimmune disease of the central nervous system. Microbial organisms may trigger the activation of CNS-specific, auto-aggressive lymphocytes either through molecular mimicry or via bystander activation. This presentation will also highlight the approaches we are taking to translate these findings to MS patients.

Saturday, December 7th Session: NEUROIMMUNO
Poster presentation

# NEUROIMMUNOLOGY OF ZBTB20 TRANSGENIC MICE

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Zinc finger- and BTB domain-containing protein 20 (ZBTB20) is a member of the BTB/POZ family of nuclear DNA-binding proteins and functions as a transcriptional repressor. In the immune system, ZBTB20 promotes plasma cell differentiation and longevity and regulates antibody response towards different stimuli.

Astrocytes are glial cells in the CNS (central neural system), responsible for the maintenance of the blood–brain barrier (BBB) integrity and form a boundary, that restricts entry of metabolites and peripheral immune cells into the CNS. ZBTB20 regulates astrocytogenesis in the developing brain and we hypothesize, that its' knockout may lead to abnormal BBB formation. This might have an impact on the organism and lead to autoimmune neuroinflammatory conditions. The purpose of this study is to examine the distribution, mitotic and secretory activity of immune cells in different compartments of the body in a transgenic mouse strain of 129/Sv ZBTB20 +/+, +/- and -/- in comparison to other mouse strains.

Saturday, December 7th Session: NEUROIMMUNO

Poster presentation

## THE ROLE OF IgG IN PATHOPHYSIOLOGY OF AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is an adult-onset disease, characterized principally by progressive degeneration of upper and lower motoneurons. Since numerous studies described perturbed processes in other cell types as well, the disease is considered multifactorial and multi-systemic. The role of the immune system in ALS pathology is still obscure, especially in the early stages, but with the disease progression, strong neuroinflammation is evident. Over the years, different groups reported antibodies reactive to various motoneuronal structures, and recent study suggested that prolonged and sustained intraperitoneal injection of ALS patients' sera induced the experimental motor neuron disease model in mice. Glycosylation patterns of ALS IgG and their potential use as biomarkers are also currently in focus. Some of the early work done by our group demonstrated that in cultured rat hippocampal neurons ALS IgG suppressed KCl-induced $[Ca2+]_i$ rise through P/Q- type calcium channels, and later on, that they increased the frequency of spontaneous excitatory postsynaptic currents. Thereafter, as our focus shifted to astrocytes, we demonstrated that ALS IgG increased the mobility of endosomes and lysosomes and induced a transient increase of intracellular calcium in cultured rat cortical astrocytes. Our current research aims at deeper understanding of functional activity of ALS IgG on the one hand and the patophysiological changes they induce in cultured neurons and astrocytes on the other. We now have evidence that astrocytes also release glutamate upon treatment with ALS IgG. In experiments with neuronal cultures it became apparent that ALS IgG directly affect spontaneous synchronized calcium activity, an effect that is missing when such calcium activity is not present (in premature neurons) or is abolished by blockers. Our research indicates probably a non- Fc receptor-mediated IgG effect, even though the experiments with fragmented IgG do not exclude the importance of Fc region for their pathological functionality.

Saturday, December 7th Session: NEUROIMMUNO

Poster presentation

# CYTOKINE GENE POLYMORPHISMS IN PATIENTS WITH CHRONIC INFLAMMATORY DEMYELINATING POLYNEUROPATHY

Emina Milošević[1], Verica Paunović[1] Irena Vuković[1], Stojan Perić[2], Ivana Basta[2], Ivo Božović[2], Aleksa Palibrk[2], Mirjana Arsenijević[2], Ivana Berisavac[2], Zorica Stević[2], Vladimir Trajković[1]

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Chronic inflammatory demyelinating polyneuropathy (CIDP) is debilitating neurological disorder caused by inadequate immune response against self-proteins present in peripheral nerves. It is the most common chronic form of immune-mediated neuropathies. Although rare, it bears considerable socio-economic burden. Mechanisms underlining the pathogenesis of this heterogeneous group of diseases include loss of immunological tolerance to myelin or axonal components, but how these diseases are triggered and why immune system reacts against these self-antigens is poorly understood.

We assessed if functional single nucleotide polymorphisms (SNPs) in the genes encoding IL-10 (rs1800871, rs3024505), TNF (rs1800629, rs361525), IL-12B (rs3212227) and IL-23R (rs11209026) associate with the occurrence of CIDP in 89 patients compared to 486 healthy controls by qPCR TaqMan assays.

We found higher proportion of allele C for SNP in the *IL10* promotor -819 C/T (rs1800871) in patients with CIDP ($p<0.05$). Distribution of genotypes for this SNP was similar in diseased and healthy people. For other investigated SNPs frequencies of alleles and genotypes were similar in CIDP cohort compared to healthy.

Studies of SNPs in CIDP are scares and this is the first study on SNPs in cytokines in these patients. Potentially it would be useful to correlate these polymorphisms with actual cytokine production and clinical course and outcomes of patients with CIDP.

Supported by Ministry of Education, Science, and Technological Development, Republic of Serbia, Grant No. 41025 and 175038

Poster presentation

## EXPRESSION OF ECTO-5' NUCLEOTIDASE ON AMOEBOID MICROGLIA DURING NEURODEGENERATION INDUCED BY TRIMETHYLTIN INDICATES ITS ROLE IN PHAGOCYTOSIS

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Microglial cells invade the brain as amoeboid precursors and acquire a highly ramified morphology in the adult brain. Also, these glial cells express all essential components of purinergic signaling system, including ecto–enzymes, which plays a major role in the regulation of phagocytosis in activated microglia during different neuropathologies. Ecto-5'-nucleotidase (CD73) is a part of ecto-enzyme chain which sequentially degrades extracellular ATP to immunosuppressive adenosine, but expression of the enzyme in different morphological stages of activated microglia is unknown. Activation of resident microglia is one of the components in the progression of trimethyltin (TMT)-induced neurodegeneration. Three week after bilaterally ovariectomy, Wistar rats were treated with a single dose of TMT (8 mg/kg, i.p) and sacrificed 2-, 4-, 7- and 21 days post-treatment. A combination of histological methods was used to determine activity and expression of CD73 in the hippocampal microglia of TMT treated animals. Microglial cells of mixed morphology gradually populated CA1 and hillar/pCA3 hippocampal sub-regions. Four days post-treatment microglia changed morphology from resting to bushy/amoeboid type as a result of TMT-induced neurodegeneration Activation of microglia during investigated timeframe was reflected as a huge increase in the cell number and the full repertoire of microglial shapes and forms, including unusual bushy and long, rod-shaped microglia. AMPase histochemistry and CD73-ir indicated reduction of staining in synaptic regions 2-d post-treatment, and after that, increase of specific labeled glial cells that infiltrated neuronal layers. From that time-point CD73 nicely depicted processes of bushy microglia, while amoeboid type completely colocalized with CD73. At day 21 post-treatment, numerous CD73+/IBA1+ amoeboid/phagocytic cells were noticed. Degree of CD73 expression correlates with morphological stage of microglial cells, suggesting the specific role of CD73 in amoeboid/phagocytic microglia. Taken together, these data show that CD73 plays a prominent role in controlling adenosine levels and thereby microglial phagocytosis.

Supported by the Ministry of Education, Science and Technological development, Republic of Serbia, Grants III41014 and OI173044

Poster presentation

# PROPRANOLOL REDUCED SEVERITY OF EAE BY INCREASING THE EXPRESSION OF Nrf2 IN MICROGLIA

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Sympathetic dysfunction was proposed to participate in development of multiple sclerosis and its animal model, experimental autoimmune encephalomyelitis (EAE). This may be linked with findings indicating that noradrenaline, the key sympathetic end-point mediator, through β-adrenoceptor exerts immunomodulatory action. Considering importance of the target tissue for the clinical outcome of EAE, the study investigated the effects of propranolol, a non-selective β-adrenoceptor blocker, on the disease severity in Dark Agouti rats. Administration of propranolol over the effector phase of EAE substantially moderated neurological symptoms of the disease. This correlated with the increased proportion of spinal cord microglia expressing CX3CR1, the crucial neuroinflammation-limiting molecule, and upregulated expression of Nrf2, the key CX3CR1 downstream target gene. Additionally, in spinal cord of propranolol-administered rats the expression of heme-oxigenase 1, Nrf2 target gene, was upregulated. Consequently, microglia from propranolol-administered rats, exhibited increased proportion of IL-10–expressing cells, but decreased those of IL-1β– and IL-23–expressing ones. Propranolol also downregulated the IL-6 and MCP-1/CCL2 expression in spinal cord. Furthermore, propranolol affecting CXCR1/Nrf2 signaling pathway enhanced microglial phagocytic/endocytic capacity and surface expression of anti-inflammatory CD163/CD83 markers. Results from *in vitro* pharmacological study examining influence of noradrenaline/propranolol on functional properties of microglia showed that microglia synthesize noradrenaline, which, in turn, through β-adrenoceptor, downregulated their Nrf2 expression, in a CX3CR1-independent manner. In accordance with microglial shift towards a more anti-inflammatory profile, in spinal cord of propranolol-administered rats was found: i) decreased infiltration with blood-borne myeloid and CD4+ T cells, and ii) reduced CD4+ T-cell reactivation/proliferation and differentiation into highly pathogenic IL-17+ cells co-producing IFN-γ and GM-CSF. The study suggests a neuroinflammation-promoting role for central noradrenaline in EAE, via β-adrenoceptor–mediated modulation of microglial Nrf2 expression. Thus, it points out to a putative target for future translational pharmacological research to optimize multiple sclerosis therapy. Funding: MPNTR RS (grant number 175050).

Saturday, December 7th Session: NEUROIMMUNO

Poster presentation

# CAN BIDERICTIONAL MODULATION OF ALPHA5 $GABA_A$ RECEPTORS RESULT IN BIDERICTIONAL CHANGES OF BEHAVIOR IN 5xFAD MOUSE MODEL OF ALZHEIMER'S DISEASE?

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Alzheimer's disease (AD) poses a major concern due to decline in cognition, mood and neurological status. As GABAergic transmission in brains of AD patients is impaired, we assessed the behavioral consequences of positive and negative allosteric modulation of $GABA_A$ receptors, elicited by MP–III–022 as PAM, and PWZ–029 as NAM, in 5xFAD mouse model of AD. Six–month old female 5xFAD transgenic (Tg) and non–transgenic (wildtype, Wt) littermates were treated with PWZ–029 (P), MP–III–022 (M) or solvent (S) for 10 days i.p. prior to behavioral testing (groups: WtS, n=16; WtP, n=15; WtM, n=15; TgS, n=11; TgP, n=12; TgM, n=10). The behavioral battery comprised of elevated plus maze (EPM), open field test (OF) and novel object recognition test (NORT). In EPM, genotype had an impact on percent of open arm entries (%OE) and percent of open arm time (%OT); transgenic mice had higher values for both parameters. MP–III–022 decreased CE and increased %OE in TgM compared to WtM, and also increased CE in WtM compared to WtS. PWZ–029 increased %OE and %OT in TgP compared to WtP. In OF, percent of peripheral distance was higher in TgM than in TgS. In NORT for long–term memory (24h), overall genotype impact was significant in time spent with old and new objects, with decreased values in transgenic mice. In TgM and WtS, number of entries into zone with new object was increased in comparison with entries into zone with old object. The old object was explored less by TgM compared to TgP and WtM groups. In Wt animals, PWZ–029 increased exploration time of the new compared to old object. Both, PAM and NAM decreased anxiety parameters in transgenic animals. On the other hand, only PAM could facilitate long–term memory in transgenic animals.

Saturday, December 7th Session: NEUROIMMUNO

Poster presentation

# THE EFFECTS OF INTERFERON-BETA TREATMENT IN A BELGRADE COHORT OF RELAPSING-REMITTING MULTIPLE SCLEROSIS PATIENTS

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Introduction: Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating and neurodegenerative disease of the central nervous system. Long-term outcome has been changed since 1993 when the interferon-beta (INF-β) was approved. The aim: The aims of the study were to determine the annual relapse rate in patients treated with INF-β in comparison with untreated patients; to estimate time to reach sustained functional neurological disability expressed in EDSS score 4.0 and 6.0, respectively in these two cohorts, and then estimating the time to secondary progressive multiple sclerosis (SP)MS. Patients and methods: In prospective cohort study, 236 patients treated with INF-β were included, and also a control group of 183 untreated with relapsing-remitting MS. Patients were observed initially for 7 years, and from this original cohort of patients, 10-year follow-up was available for 233 treated patients and 131 untreated. Median follow-up time was 9.7 years. Results: Treatment with INF-β statistically significantly delayed time for reaching all three outcomes (SP, EDSS score 4.0 and EDSS score 6.0). The time for reaching SP in the cohort of treated patients was 9.7 years, and in untreated 7.8 years since baseline. The time for reaching EDSS score 4.0 in the treated patients was 8.7 years from the baseline, and in untreated 7.1 years. Also, the time for reaching EDSS score 6.0 was extended; in the treated patients 9.8 years since baseline, and for untreated 8.8 years. INF-β was associated with a statistically significantly lower risk of developing SP in comparison with the cohort of untreated patients (HR=0.22), and also, there was a significant difference in the achievement EDSS score 4.0 and 6.0 ($p<0.001$), in a benefit of treated patients (HR=0.40 and HR=0.27, retrospectively). Conclusion: Treatment with INF-β has a statistically significant benefit on all three outcomes, and this therapy may also have a statistically significant effect on the long-term outcome in patients with MS.

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Poster presentation

# THE ANALYSIS OF LABORATORY DATA FROM PATIENTS WITH NEUROMYELITIS OPTICA SPECTRUM DISORDER

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The aim of this study was to analyze laboratory data from patients with neuromyelitis optica spectrum disorder (NMOSD).

Demographic, clinical, and laboratory data, such as, first performed: routine cerebrospinal fluid findings, serum anti-aquaporin 4 (AQP4), antibodies against other serum autoantibodies, were evaluated in 74 Caucasian NMOSD patients, from the Clinic of Neurology, CCS, Belgrade, who fulfilled the 2015 diagnostic criteria. Serum AQP4 IgG (NMO-IgG) was tested using a commercial cell-based indirect immunofluorescence assay (Euroimmun AG). The result was considered to be positive if tested positive at the dilution of 1:10. In addition, antibodies against myelin-oligodendrocyte (MOG-IgG) in serum were tested in a subset of 47 randomly selected NMOSD patients for whom enough sample volume was available, using a live cell-based assay. Antibodies against myelin-oligodendrocyte (MOG-IgG) in serum were tested in a subset of 47 NMOSD patients. Analysis of results showed that 89.2% of patients were tested NMO-IgG positive by our method. Median NMO-IgG titer was 1:640 (range, 0-1:40960). In addition, in the subgroup of 47 NMSD patients who were tested for MOG-IgG, 2 out of 7 patients tested to be NMO-IgG negative by the live cell based assay we found MOG-IgG to be positive. The analysis of first ever performed routine CSF findings showed a median CSF protein level of 0.45g/L (range, 0.28-2.23 g/L), median CSF cell count of 3.5/μL (range, 0-378 mononuclear cells/μL; CSF cell phenotype: lymphocytes, granulocytes, macrophages) and the presence of intrathecal synthesis of CSF oligoclonal IgG bands in 16 out of 66 (24.2%) patients in whom CSF analysis was performed.

In conclusion, laboratory findings have a significant role in the differential diagnosis of NMOSD. Additionally, it has to be emphasized that the high proportion of anti-AQP4 antibodies in our patients, implicate absolute necessity of determination of anti-AQP4 antibodies status for diagnostic purposes in NMOSD.

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Poster presentation

## THE EFFECTS OF FOOD RESTRICTION ON ANXIETY LEVEL AND DOPAMINERGIC SYSTEM DURING AGING IN MALE WISTAR RATS

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Aims: Food restriction (FR) is well known as an environmental intervention efficient in delaying aging and age-related disorders. Important role in the regulation of food intake plays the gut-brain dopamine (DA) axis. Dopamine is a neurotransmitter involved in regulation of brain's rewarding and pleasure centers, who's signaling is indispensable to survival and maintenance of eating patterns. Reversely, reduced food intake affects DA circuits and behaviors controlled by DA, including anxiety. Herein we investigated mechanisms through which FR affects anxiety and the role of dopaminergic system in this process. Methods: 60% FR of various onset and duration (FR1, FR2 and FR3) was implemented as a feeding regime for aging male Wistar rats. Open field test and light-dark box were used to investigate effects of age and food restriction on anxiety-like behavior. Western blot and PCR were used to determine the changes at the transcriptional and translational level. Results: Open field test showed an increased general activity of animals under FR1 in comparison to the controls, while FR2 and FR3 seemed to have deleterious effect on anxiety level. Light-dark box confirmed deleterious effect of FR2 and FR3 regimens. Changes detected on behavioral level were accompanied with the specific changes in the level of dopaminergic receptors. Conclusions: Our results showed that food restriction is not universally beneficial, but depends on age when implemented. We showed that FR-induced effects can vary from anxiolytic to anxiogenic, while the components of DA circuits in the brain show region-specific response to FR.

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Poster presentation

## INTERMITTENT FEEDING EXACERBATES INFLAMMATION IN MOUSE MODEL OF ALZHEIMER'S DISEASE

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Numerous beneficial effects of food restriction (FR) on aging and age-related pathologies are well documented. Neuroprotective effects of both daily calorie reduction (CR) and intermittent feeding (IF) were also described in animal models, reflected by reduced levels of proinflammatory cytokines, reactive oxygen species, and increased insulin sensitivity. In the present study, the effects of IF were examined in 5XFAD transgenic mice, a commonly used transgenic mouse model of Alzheimer's disease (AD). Intermittent feeding regimen was introduced to transgenic female mice at the age of 2 months and the effects on amyloid-β (Aβ) accumulation, gliosis, synaptic plasticity, and blood-brain barrier breakdown were analyzed in cortical tissue of 6-month-old animals. Surprisingly, significant increase of inflammation in the cortex of 5XFAD fed EOD mice was observed, reflected by the expression of microglial and astrocytic markers. This increase in reactivity and/or proliferation of glial cells was accompanied by an increase in proinflammatory cytokine TNF-α, p38 MAPK and EAAT2, and a decrease in GAD67. NMDA receptor subunit 2B, related to glutamate excitotoxicity, was increased in the cortex of 5XFAD-EOD mice indicating additional alterations in glutamatergic signaling. Furthermore, 4 months of EOD feeding regimen had led to synaptic plasticity proteins reduction and neuronal injury in 5XFAD mice. However, EOD feeding regimen did not affect Aβ load and blood-brain barrier permeability in the cortex of 5XFAD mice. Collectively, the present data demonstrate that EOD feeding regimen exacerbates Alzheimer's disease-like neurodegenerative and neuroinflammatory changes irrespective of Aβ pathology in 5XFAD mice.

Poster presentation

## CONCOMITANT UP-REGULATION OF ECTO-5' NUCLEOTIDASE (CD73) AND PRO-INFLAMMATORY CYTOKINES IN NEUROINFLAMMATORY MODEL OF HIPPOCAMPAL INJURY INDUCED BY TRIMETHYLTIN

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Introduction – Ecto-5' nucleotidase/cluster of differentiation 73 (CD73) is a membrane enzyme with active site facing extracellular compartment. It is the last and rate-limiting enzyme of the purine catabolic pathway, catalyzing dephosporylation of extracellular adenosine 5'-monophosphate to adenosine. Adenosine is potent homeostatic regulator in central nervous system (CNS) involved in numerous processes such as control of cell growth, cellular differentiation and an important regulator of inflammatory response. Neuroinflammation is defined as an inflammatory process within CNS. This inflammation is mediated by various cytokines, chemokines, reactive oxygen species and secondary messengers. The duration and degree of neuroinflammation depends on the context, duration and course of the primary insult. One of the validated models for obtaining a hippocampal neurodegeneration followed by neuroinflammation and gliosis is trimethyltin (TMT) intoxication. Therefore, we used TMT-intoxicated animals to study temporal expression of CD73 and main pro- and anti-inflammatory cytokines.

Material and methods - Two-month-old female Wistar rats were bilaterally ovariectomized. Three week after surgery they were treated with single i.p. dose of TMT (8 mg/kg) and sacrificed 7 and 21 day posttreatment. Brains were isolated using TRI-reagent and processed for real-time PCR analysis. Results – Seven days after intoxication, there was a significant increase in IL-6, TNF-α and iNOS but also in CD73 expression, while 21 days after exposure increase in relative gene expression was notices in IL-1β, IL-6, IL-10 and iNOS followed by continuous increase in CD73 expression. Conclusion – Temporal expression of pro- and anti-inflammatory cytokines clearly revealed and active and ongoing neuroinflammation. Increase in CD73 expression in examined timeframe could be put in perspective of its anti-inflammatory role. Since adenosine represents a potent regulator of inflammation and CD73 is its main generator, significance of its increase could lead to resolution of neuroinflammatory events caused by TMT-induced neurodegeneration.

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Poster presentation

# TRANSCRIPTOMICS OF NEUROINFLAMMATION IN ALS SOD1G93A TRANSGENIC RATS

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease with no cure at present, characterized by degeneration of motor neurons in the spinal cord, brainstem and cortex, paralysis and muscle waisting. Proposed mechanisms of ALS pathophysiology and progression include neuroinflammation, oxidative stress, mitochondrial and endoplasmic reticulum dysfunction, protein aggregation, impaired axonal transport, intracellular ion misbalance and glutamate excitotoxicity. The aim of the present study was to identify differentially expressed genes (DEGs) in the spinal cord of non-transgenic (control) rats and in pre-symptomatic and symptomatic ALS SOD1G93A transgenic rats, using microarray analysis. A total of 166 deregulated mRNAs have been identified and a list of the significantly modulated genes has been annotated by means of gene ontology. The main pathways revealed include chemokine and B cell receptor signaling pathways, complement and coagulation cascade and cytokine-cytokine receptor interactions. Immune defense and inflammatory response genes were the mostly upregulated in our ALS model. In general, the following genes were upregulated: tumor necrosis factor alpha induced protein 6 (TNFAIP6) 9 fold, and apolipoprotein B mRNA editing enzyme catalytic polypeptide 1 (APOBEC1) 7 fold, while the complement component 1 and 3 (C1, C3), transforming growth factor beta 1 (TGFB1) and its receptor (TGFBR1), as well as FYN binding protein (FYB) and B cell linker (BLNK) were upregulated more than 2 times. In order to confirm the transcriptome data, expression levels of several genes (APOBEC1, FYB, BLNK) were also determined by real-time PCR and the results were matching. Our findings provide evidence of the genes playing a role in ALS chronic inflammation.

# FLAXSEED OIL MODULATES MICROGLIA ACTIVATION IN THE HIPPOCAMPUS OF OVARIECTOMIZED RATS FOLLOWING TRIMETHYLTIN INTOXICATION

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Flaxseed (FS, *Linum usitatissimum*) poses well-known health benefits due to its high concentrations of ω-3 fatty acid: α-linolenic acid (ALA), short chain polyunsaturated fatty acids, fibers, proteins, antioxidants and phytoestrogens. An increasing number of postmenopausal women start to take FS as phytoestrogen-rich foods to prevent the risk of breast cancer, osteoporosis and some menopausal symptoms. However, role of FS in regulation of neuroinflammation is less understood. Microglial cells are resident immune cells of the central nervous system (CNS) which respond rapidly to the injury by shortening and thickening of processes and adoption of amoeboid morphology, migrate to the site of injury, and clear cellular debris. Extracellular ATP/ADP and P2 receptors are essential for the microglial activation. Thus, we examined the ability of repeated flaxseed oil (FSO, 1 ml/kg, s.c.) pretreatment to modulate activation of microglia in the hippocampus of ovariectomized female rats (OVX) induced by trimethyltin intoxication (TMT, 8mg/kg, i.p.). Seven days post-TMT intoxication enhanced total ADPase staining intensity and immunoreactivity of microglia-specific protein IBA-1 increased. Microglial activation has been manifested as significant increase in number of microglial cells, due to migration and/or proliferation of resident microglia. Observed IBA-$1^+$ cells displayed heterogeneous morphologies which gradually populated CA1 and hillar/pCA3 hippocampal sub-regions. We observed: ramified, hyper-ramified, bushy, amoeboid, and rod microglial phenotypes. Some of these phenotypes like hyper-ramified and bushy have been referred to as primed and reactive/activated, respectively. Repeated FSO pretreatment prevented overall increase in ecto-ADPase activity and IBA-1-ir cells observed after TMT. At phenotypic level microglial population exhibited ramified but also occasional hyper-ramifide phemotype with enlarged cell bodies in CA1 region, while in hillar/pCA3 region clusters of these cells were observed. Collectively, our findings suggest a novel FSO-mediated neuroprotective effect *via* modulation of microglia activation stage in the brain. Acknoledgement. Study was supported by MPNTR, Republic of Serbia, grants OI 173044 and III 41014.

Saturday, December 7th Session: NEUROIMMUNO

Poster presentation

## EXPRESSION OF AUTOPHAGY-REGULATING GENES AND CYTOKINES IN PATIENTS WITH GUILLAIN-BARRÉ SYNDROME

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Guillain-Barré syndrome (GBS) is an immune-mediated neuropathy caused by inadequate immune response against peripheral nerve proteins. Though rare, GBS bears a large socio-economic burden, since it requires an expensive immunotherapy and may cause a long-term disability. The underlying cause of immune system reaction against self antigens in GBS is unknown. Maintenance of tolerance depends on immune cell homeostasis which includes autophagy - autodigestion/recycling of organelles and macromolecules. Although autophagy is altered in animal models of GBS and drugs targeting autophagy are clinically approved, available, safe and less expensive than the conventional therapy, there are no data about autophagy in human GBS. Therefore, our primary objective is to correlate the expression of authophagy markers/regulators in immune cells with clinical and immunological parameters of GBS patients. To date, we have analyzed the expression of eighteen genes involved in autophagy regulation and cytokines related to the disease (IFN-γ, IL-1 and IL-10) in peripheral blood mononuclear cells (PBMC) from nineteen GBS patients, therapy naïve, and twenty non-diseased control subjects. The RT-qPCR analysis revealed that PBMC of GBS patients had statistically significantly higher mRNA expression of GABARAP, ATG7, ATG5, ATG7, VPS34 and TFEB, proteins involved in autophagy regulation, as well as IL-10, a well-known anti-inflammatory cytokine. The mRNA levels of other investigated autophagy-regulating genes and cytokines did not significantly differ between the two groups. However, the GABARAP, ATG7, ATG5, ATG7, VPS34, TFEB and IL-10 mRNA expression did not correlate with age, severity of disease or other clinical parameters. These results indicate autophagy may have an impact on GBS pathogenesis or disease propagation, however further in-depth research is required to reveal its role in GBS.

Poster presentation

## ENVIRONMENTALLY RELEVANT EXPOSURE TO CADMIUM AND HEALTH RISKS: SKIN AS TARGET ORGAN

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Adverse effects of non-occupational exposure to cadmium (Cd) are increasingly acknowledged. Using a rat model of oral Cd exposure in drinking water we have shown that skin is a target for this metal. Due to contribution of individual variability to the intensity of cadmium toxicity, dermatotoxicity of two environmentally relevant Cd doses (5 and 50 ppm) was examined in individuals of two rat strains, Albino Oxford (AO) and Dark Agouti (DA), which differ in response to chemicals. A dose-dependent accumulation of Cd in the skin/epidermal cells was noted in both strains, and although there were no strain differences in the Cd accumulation, the degree of skin response to the metal differed. Signs of skin damage were evident in both strains, but response to injury was more pronounced in DA. Individuals of DA rats responded by an increase in the levels of antioxidant defense enzymes in the skin already at lower dose, in contrast to AO (which reacted to higher dose solely), implying higher sensitivity of DA strain to Cd-induced toxicity. Epidermal cells from both strains developed stress response, however increased GSH, and higher metallothionein/MT-1 and MT-2 mRNA, Nrf2 protein, apoptosis, Ahr and Cyp genes in AO, depicting this strain`s ability to better defend against Cd insult. Epidermal cells` IL-1β, TNF and IL-6 response was induced by Cd in DA, while pro-inflammatory cytokine production was unchanged in AO (though increased following stimulation with *S. epidermidis*), with increased IL-10 as a possible underlying mechanism. T cells from non-exposed rats produce more IFN-γ and IL-17 in co-culture with epidermal cell from Cd–exposed DA rats what strengthens the view that this strain is more prone to metal's dermatotoxicity. These data give a new insight into repercussion of genetic variability to toxicity of cadmium acquired by the skin via gut, bearing relevance for variations in the link between dietary cadmium and inflammation-based skin pathologies.

Supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant #173039.

Saturday, December 7th Session: MISCELLANEOUS

Poster presentation

# ENVIRONMENTALLY RELEVANT EXPOSURE TO CADMIUM AND HEALTH RISKS: INVOLVEMENT OF ARYL HYDROCARBON RECEPTOR

Jelena Kulas[1], Dina Tucovic[1], Milica Zeljkovic[1], Dusanka Popovic[1], Aleksandra Popov Aleksandrov[1], Milena Kataranovski[1], Ivana Mirkov[1]

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Cadmium (Cd) is a heavy metal widely spread in the environment and significant water and food contaminant. This metal exerts toxic effects in various tissues thus representing great threat to human health, and our previous study showed that oral consumption of Cd (in water for 30 days) increased the metal deposition and exerted immunomodulatory effects in lung leukocytes. Although the most studied mechanisms of Cd toxicity include oxidative stress and inflammation, recent studies have indicated that this metal can activate aryl hydrocarbon receptor (AHR) and exert effects on AHR-regulated genes (i.e. CYPs). AHR represents a link between environmental toxicants and immune response as high receptor expression is noted in immune cells and barrier tissues, thus the aim of presented study was to investigate if activation of AhR by Cd is associated with metals' immunomodulatory effects. Treatment of lung leukocytes with Cd *in vitro* (non-toxic doses) caused an increase in mRNA levels for AHR, CYP1B1 and CYP1A1, but co-treatment with metal and AHR antagonist CH223191 indicated that higher Cd doses (5 and 10 μM) can activate CYPs directly while a lower dose (1 μM) exerted effects on CYPs expression through activation of AHR. Low Cd dose induced increased production of IL-6 and decreased TNF and IL-1β by lung leukocytes, compared to controls. Gene expression data revealed unchanged mRNA for IL-6, decreased TNF, but increased IL-1β. Lower IL-1β protein level despite increased mRNA, was a consequence of decreased mRNA for NLRP3, a component of inflammasome that is involved in processing of pro-IL-1β in IL-1β. All noted effects were abolished in the presence of CH223191. Data obtained indicate that immunomodulatory effects of low Cd dose are mediated through AHR activation.

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Saturday, December 7th Session: MISCELLANEOUS

Poster presentation

## ATP DEPENDENCY OF ORIC, VRAC-LIKE CURRENT FROM FILAMENTOUS FUNGUS *Phycomyces blakesleeanus*

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Volume regulated anionic current (VRAC) controls regulatory decrease in cellular volume. It is mediated by LRRC8A-E channel proteins, present ubiquitously in chordate lineage. Besides VRAC being involved in basic cellular functions, it is considered especially important for lymphocyte and macrophage physiology, the notion exemplified by the fact that the only known human LRRC8 mutation, identified from a patient with agammaglobulinemia, exerts a dominant negative effect on T- and B-cell development in mice. VRAC channel permeates $Cl^-$ and other anionic species (bicarbonates, sulphates, nitrates) as well as large organic osmolites (glutamate, aspartate, taurine, ATP); it's activity requires ATP binding from intracellular side; it can be activated or modulated by G proteins, ROS, reduction of intracellular ionic strength, AKT and RAS kinase, among other things. Here, we present patch-clamp analysis of a VRAC-like current from unexpected source, the membrane from filamentous fungi *Phycomyces blakesleeanus*, that we named ORIC. We have previously shown that ORIC has following VRAC properties: activation by hypoosmotic stimulus; ion selectivity and ion channel permeation sequence; biophysical properties of voltage dependent inactivation and activation; activation by GTPγS. Continuing our characterization of ORIC, here we show that, same as reported for VRAC by others, ORIC is ATP-dependent, as well as blocked by ATP from extracellular side. Namely, ORIC run-down is prevented by inclusion of ATP or non-hydrolysable ATP analog in the patch pipette; flavonoids genistein and quercetine reduce ORIC; ATP (500 μM) on the extracellular side blocks ORIC. The studying of ORIC in our fungal model system, has a unique advantage, as ORIC represents the naturally occurring osmotically activated current, with all regulatory elements intact, while conveniently isolated for observation, since ORIC is the most dominant current under hypoosmotic conditions. The extent of functional and, possibly structural, similarity between ORIC and VRAC needs yet to be determined.

Saturday, December 7th Session: MISCELLANEOUS

Poster presentation

# EFFECTS OF DIETARY SEED MIXTURE SESAME/PUMPKIN/FLAX ON INLAMMATORY MARKERS IN PATIENTS ON HEMODIALYSIS

Marija Takić[1], Danijela Ristić-Medić[1], Tamara Popović[1], Gordana Peruničić-Peković[2], Zorica Rašić-Milutinović[3], Aleksandra Arsić[1], Marija Glibetić[1]

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Chronic inflammation is found in a great proportion of chronic kidney disease patients on hemodialysis. As some food are benefical for human health, possibly because of their anti-inflammatory effects, we studied effects of dietary consumption of milled sesame/pumpkin/flax seed mixture on inflammatory markers in patients on hemodialysis. Thirty hemodialysis patients consumed 30g of milled sesame/pumpkin/flax (6g/6g/18g, respectably) added to their habitual diet for 12 weeks. Their levels of TNF-alpha, hs-CRP and IL-6 were determined at baseline and after treatment. The mean level of TNF-alpha was 1.86±0.90 mU/L, for IL-6 2.63±1.45 pg/ml and hs-CRP 6.26±4.12 mg/L. After treatment, levels of inflammatory markers TNF-alpha, hs-CRP and IL-6 were significantly decreased compared to baseline values ($p<0.001$, for all parameters). Results of this study indicate that seed mixture sesame/pumpkin/flax added to habitual diet could improve inflammatory status of chronic kidney disease patients on hemodialysis as all studied markers of inflammation were significantly decreased after treatment.

Saturday, December 7th Session: MISCELLANEOUS

Poster presentation

# MIF REGULATES IL-8 EXPRESSION IN HTR-8/SVneo CELL LINE

Milica Jovanović Krivokuća, Ivana Stefanoska, Aleksandra Vilotić, Ljiljana Vićovac.

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Trophoblast cells represent specific placental cells which invade into uterine tissue to establish and maintain a successful pregnancy. Along the invasive pathway of differentiation trophoblast comes into close contact with various maternal cell types including decidual stromal cells and various immune cells. Multiple factors produced by both trophoblast and maternal cells regulate trophoblast invasion process, including IL-1β, IL-6, IL-8, LIF, EGF, HGF and many others.

Macrophage migration inhibitory factor (MIF) is a multifunctional cytokine that participates in both inate and adoptive immunity. It is abundantly present at the feto-maternal interface, and expressed by trophoblast and maternal cells. We have previously shown that this cytokine regulates trophoblast cell invasion and that it might participate in trophoblast response to infection. Data from the literature implicate MIF as an upstream regulator of proinflamatory cytokines. For that reason, a possibility that MIF could regulate the expression of cytokines known to participate in trophoblast invasion was investigated here. HTR-8/SVneo cells were used as the model for extravillous trophoblast. The expression of endogenous trophoblast MIF was attenuated by specific small interfering RNAs (siRNA) for 72h. MIF activity was also blocked in a separate treatment group by a small chemical inhibitor ISO-1. The expression of cytokines was assessed at mRNA level by qPCR and at the protein level by flow cytometry. The results obtained show that IL-8 is significantly reduced after silencing of mRNA or blocking of MIF activity by ISO-1.

It can be concluded that MIF regulates IL-8 expression in HTR-8/SVneo cell line, which may be important for trophoblast invasion and/or response to infection.

## Poster presentation

### PROBIOTIC ACTIVITY AND MICROENCAPSULATION OF *Lactobacillus reuteri B2* ISOLATED FROM FECES OF C57BL/6 MICE

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The major parts of the commensal microbial flora of the human gastrointestinal tract (GIT) belong to the lactic acid bacteria (LAB) that are frequently used as probiotics. Probiotics must survive passage through the upper GIT and reach its site of action alive, where they have to maintain their stability, viability and function. Preparation of various biopolymer-based carriers and microencapsulation become inevitable part of probiotics-focused researches. In this study, we assessed probiotic activity of *Lactobacillus reuteri* B2 selected from the panel of LAB isolated from the feces of C57BL/6 mice. *L. reuteri* B2 *was* evaluated as potential probiotic and its microencapsulation with alginate-based materials were performed. We hypothesized that if *L. reuteri* B2 in the free form can survive all conditions in the GIT then the usage of the appropriate biomaterials for microencapsulation would improve its viability and stability in GIT. Consequently, there has been assessed *L. reuteri* B2 in free and in microencapsulated form, *in vitro*, in the culture of epithelial cells. High survival rate of *L. reuteri* B2 at low pH (2.0- 4.0) and in the presence of the bile salts at concentrations up to 0.30% imply that it can survive harsh conditions within GIT. Likewise, *L. reuteri B2* strong antimicrobial activity toward pathogen species on which this strain has been assessed. Furthermore, testing of the alginate-based polymers revealed no negative impact on the viability of epithelial cells. Results obtained from this study highly encourage further research on the impact of alginate-encapsulated *L. reuteri* B2 in physiological conditions as well as for the prevention or treatment of some pathological states. Additionally, demonstrating extended viability of encapsulated probiotics *in vivo* will justify scaling up the encapsulation process for commercial application. Acknowledgment: The authors would like to acknowledge the financial support of the Ministry of Education, Science and Technological Development, Government of Republic of Serbia, projects: OI 176018 and OI172049.

Poster presentation

## SEXUAL DIMORPHISM IN THYMIC SENESCENCE

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The study showed sexual dimorphism in the kinetics of thymic involution in Dark Agouti rats, so in 24-month-old males prominent thymic fibro-adipose degeneration was found, whereas fibrous changes dominated in thymi of age-matched females. This dimorphism reflected sex-specific constellation of age-related alterations in the expression of the key proadipogenic factors (xanthine oxidase-induced PPARγ, STAT3, the transcription factor controlling PPARγ downstream adipocyte-differentiation-related gene expression, and IL-6) and TGF-β, the key pro-fibrinogenic factor. The age-related epithelial-mesenchymal transition in thymi of Dark Agouti rats was accompanied by decline in thymopoiesis mirrored in decrease in the frequency of the most mature CD4+CD8-/CD4-CD8+ TCRαβ$^{high}$ thymocytes and CD4+ and CD8+ recent thymic emigrants in peripheral blood (PB). This was more prominent in males than in females. Irrespective of sex, differentiation/maturation "block" leading to accumulation of the least mature CD45RC+CD2-CD4-CD8- thymocytes, accompanied by decline in the frequency of descendant double positive (DP) TCRαβ$^{-}$ ones was observed with aging. This was followed by opposing changes in the efficacy of positive/negative selection in males and females, which was related to sex-specific alterations in thymic expression of Nur77, a nuclear receptor involved in negative selection, and surface density of CD90 (negative regulator of thymocyte-selection threshold) on thymocytes undergoing selection. Moreover, compared with old females, in age-matched males CD4+CD8-/CD4-CD8+ TCRαβ$^{high}$ thymocyte ratio was shifted towards the latter. However, CD4+/CD8+ ratio in PB was skewed towards CD8+ T cells in both sexes. Irrespective of sex, with aging the frequency of CD4+CD25+Foxp3+ thymocytes diminished, but that of CD4+CD25+Foxp3+ T regulatory cells (Tregs) in PB increased, most likely due to enhanced expansion of "induced" Tregs. Collectively, the study i) indicates necessity of sex-specific approaches in designing thymus-rejuvenating strategies and ii) warns that changes in the PB T-cell compartment do not necessarily reflect sex-based differences in T-cell generation in thymus. (Funding: project 175050 MNTR RS)

Poster presentation

## MELDONIUM PREVENTS ACUTE ISCHEMIA/REPERFUSION INDUCED-RENAL CELLS DEATH IN RATS

Siniša Djurašević[1], Maja Stojković[2], Ljiljana Bogdanović[2], Ilijana Grigorov[3], Desanka Bogojević[3], Nebojša Jasnić[1], Predrag Vujović[1], Tamara Dakić[1], Zoran Todorović[2,4] and Jelena Djordjević[1]

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Acute renal ischemia/reperfusion (I/R) is a temporary restriction of kidney blood supply, followed by blood flow restoration and re-oxygenation. During I/R, decreased oxygen supply disturbs ion transport, intracellular ATP, calcium and pH levels, and numerous signalling pathways. Upon reperfusion, a restoration of oxygen level rises a reactive oxygen species generation, cytokines and chemokines release from activated tissue-resident macrophages, and infiltration of pro-inflammatory neutrophils into ischemic tissues. All these changes result in cell swelling and rupturing, and consequent necrotic or apoptotic cell death. Meldonium is an anti-ischemic drug clinically used to treat myocardial and cerebral ischemia, which acts by shifting energy production from fatty acid oxidation to glycolysis. We investigated the effects of a 4-week meldonium pre-treatment with 300 mg/kg b.m./day of rats subjected to a well-established experimental model of renal I/R, with ischemia lasting for 45 minutes, followed by 4 hours of reperfusion. The degree of apoptosis and necrosis was evaluated by measuring renal pro-apoptotic Bax and anti-apoptotic Bcl-2 ratio, serum and kidney levels of necrotic marker - high mobility group box 1 protein (HMGB1), together with the kidney histology analysis. Our results showed that apoptotic and necrotic cell death occur simultaneously under I/R conditions, judging by the renal Bax/Bcl2 ratio rise (2.7-fold), increase in serum (22%) and renal (30%) levels of HMGB1, as well as severe tubular necrosis with dilatation of the tubular structure, cast formation, tubular lumina dilatation, brush border reduction, and loss in some renal areas cells. Meldonium pre-treatment reduced the elevated Bax/Bcl2 ratio by 35%, as well as the serum and renal HMGB1 levels by 20% and notably diminished histological evidence of renal I/R necrotic injury, especially regarding tubular structures. These findings proved that meldonium protects renal cells against I/R-induced necrosis and apoptosis.

Saturday, December 7th Session: MISCELLANEOUS

Poster presentation

## COPD PATIENTS EXHIBIT LOWER IgG SIALYLATION LEVEL IN COMPARISON TO ACUTE BRONCHITIS PATIENTS

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Chronic obstructive pulmonary disease (COPD) is characterized by decreased air flow in the airways leading to shortness of breath, coughing and wheezing. Inflammation and oxidative stress play an important role in the pathophysiology of COPD. One of the primary goals of therapy is to reduce the number and frequency of exacerbations. Inhaled corticosteroids and phosphodiesterase inhibitors are used in addition to bronchodilators in patients with high rates of exacerbations. However, the use of inhaled corticosteroids can cause pneumonia. Mucolytics play an important role in the prevention of exacerbations. The goal of this work was to analyze immune parameters at the onset of disease/exacerbation and upon one month of supplementation with N-Acetyl cysteine (NAC) or propomucil (NAC with propolis) in age matched acute bronchitis patients and COPD patients. Cytokines: IL-17, IL-6, and IL-8, were analyzed from patients' plasma with commercial ELISA kits. IgG sialylation was assessed in ELISA with Protein G for IgG capture and SNA I – a lectin specific for sialic acid attached to terminal galactose with α-2,6 glycosidic bond. We were not able to detect significant differences in plasma cytokine levels irrespective of the patient group or treatment. No difference was found in IgG sialylation upon treatment. However, COPD patients had significantly lower level of IgG sialylation compared to acute bronchitis patients. The obtained results indicate that the influence of NAC on the immune system, with or without propolis can not be evaluated by assessing plasma cytokine levels, as the effects might be localized. While there were no differences in sialylation related to treatment, the obtained lower level of IgG sialylation is an indicator of increased level of chronic inflammation present in COPD patients, which is in accordance with literature data. As no differences were noted in this group within one month this likely represents a permanent state.

# Saturday, December 7th Session: MISCELLANEOUS

## Poster presentation

## Val158Met CATECHOL O-METHYL TRANSFERASE POLYMORPHISM AND INCREASED PROINFLAMMATORY CYTOKINE GENE EXPRESSION AS PREECLAMPSIA RISK FACTORS

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Introduction: Poor placentation leads to different pathophysiological mechanisms that contribute to preeclampsia development. Single nucleotide gene polymorphism (Val158Met) for catechol O-methyl transferase (COMT) is related to inadequate spiral artery remodeling during placentation. Endothelial dysfunction may be caused by disturbed immunology response and increased production of proinflammatory cytokines. Aims: To determine genotypes and alleles frequencies for Val158Met COMT polymorphism and proinflammatory cytokines relative gene expression in preeclampsia and control group, and to analyze potential relationship between these parameters. Materials and methods: Case-control study included 50 preeclampsia patients and 50 healthy pregnant women. Groups were matched for age, parity, gravidity, singleton pregnancy and gestational age at sampling. Polymerase Chain Reaction/Restriction Fragment Length Polymorphism technique was used to determine polymorphism in COMT gene. Real time Polymerase Chain Reaction was used to determine relative gene expression of proinflammatory cytokines. Appropriate statistical tests were applied for data analysis.

Results: Homozygous mutation for Val158Met COMT gene polymorphism was more frequent in the study than in the control group (P=0.002) and carried a 3.7-fold risk increase for preeclampsia. Variant allele (A) was related to significantly higher risk for development of this hypertensive disorder. Relative mRNA expression of tumor necrosis factor (TNF)-α and interleukin (IL)-1β was significantly higher in preeclampsia than in healthy pregnant women (P=0.006 and P=0.005, respectively). Analysis of all examined patients revealed higher TNF-α expression in the presence of COMT polymorphism (P=0.012). Conclusions: Val158Met COMT gene polymorphism represents a risk factor for preeclampsia development. Preeclampsia was associated with higher expression of TNF-α and IL-1β. Decreased COMT activity is related to marked inflammatory response during pregnancy. Key words: Pre-Eclampsia; Catechol O-Methyltransferase; Polymorphism, Genetic; Tumor Necrosis Factor-alpha; Interleukin-1beta; Interleukin-6; inflammation.

Poster presentation

## REGULATORY CYTOKINES PRESCRIBE THE PROCESS OF PSEUDOEXFOLIATION GLAUCOMA PROGRESSION

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Background: Pseudoexfoliation syndrome (XFS) is an age-related systemic disorder with ocular manifestations. Pseudoexfoliation is the most common cause of pseudoexfoliation glaucoma (XFG) development. The aim of this study is to reveal the participation of regulatory cytokines within the process of pseudoexfoliation (PEX) production and accumulation.

Methods: Our study included 160 patients referred to cataract surgery with early and late stage of XFS or XFG. Humour and serum levels of cytokines TGF-β, PDGF, EGF, IGF, IL-8 and ITAC were measured in a sample with high sensitivity enzyme-linked immunoabsorbent assay (ELISA) kit.

Results: Our results indicate that profibrotic action induced by increasing TGF-β and PDGF locally activates fibrous tissue production in the early XFS with prolonged effect of PDGF (late XFS) and finally (XFG stage) it is dominantly controlled by EGF and IGF. ITAC overrides angiogenetic effects of IL-8 in XFG. Conclusions: Based on our findings the local chronic inflammation in the eye is accompanied by the action of different profibrotic cytokines (TGF-β, PDGF, EGF, IGF and IL-8). Accumulated PEX material in all parts of the eye and decreased blood flow cause XFG development without vasculogenesis and angiogenesis due to the increased level of ITAC (humor aqueous and sera) in XFG, despite to local oxidative stress.

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Saturday, December 7th Session: MISCELLANEOUS

Poster presentation

## GALECTIN-3 INFLUENCES TROPHOBLAST CYTOKINE MILIEU

Milica Jovanović Krivokuća, Ivana Stefanoska, Aleksandra Vilotić, Žanka Bojić-Trbojević, Ljiljana Vićovac

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Galectins (gals) are β-galactoside binding proteins abundantly expressed at the feto-maternal interface and implicated in various processes during embryo implantation and placentation. Extravillous trophoblast cells express gal-1, gal-3 and gal-8. Our group has recently shown that gal-3 acts as a modulator of trophoblast invasion *in vitro*. This study aimed to investigate whether trophoblast gal-3 could act as a modulator of cytokines which are known to participate in trophoblast invasion.

In this study extravillous trophoblast cell line HTR-8/SVneo was used. Expression of gal-3 was attenuated using specific small interfering RNAs (siRNAs), and the silencing efficiency was assessed by qPCR and Western blot. The expression of cytokines known to regulate trophoblast invasion IL-1β, IL-6 and IL-8 was studied at mRNA level by qPCR and at the protein level by flow cytometry. The data show that the expression profiles in gal-3 silenced cells differ at both mRNA and protein level.

These results indicate that endogenous trophoblast gal-3 could act as a regulator of cytokine expression in HTR-8/SVneo cells, which could further reflect on trophoblast invasive capacity and other trophoblast cell functions.

Sunday, December 8th

Preliminary lecture

# T CELL IMMUNITY DURING SEPSIS-INDUCED IMMUNOPARALYSIS STATE – A FEW SHORT STORIES

Vladimir P Badovinac[1, 2, 3]

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Sepsis results in a deluge of both pro- and anti-inflammatory cytokines leading to lymphopenia and chronic immunoparalysis. The pathogenesis of sepsis is complex and variable, depending on the pathogen load and virulence, host comorbidity, genetic and epigenetic factors, environment, and the amount of time passed after the onset of the infection, with distinct phases of the immunological responses defined at local and systemic levels. Sepsis induced long-lasting immunoparalysis is, in part, defined by impaired CD4 and CD8 αβ T cell responses in the post-septic environment. The dysfunction in T cell immunity affects naïve T cell compartment (primary T cell responses) as well as infection- or vaccine-induced effector and memory T cell responses leading to increased susceptibility to secondary bacterial and viral infections and tumor development. While the sepsis-induced severe and transient lymphopenia is a contributory factor to diminished T cell immunity, T cell-intrinsic and -extrinsic factors/mechanisms also contribute to impaired T cell function. I will discuss the current knowledge obtained on novel experimental models of how sepsis quantitatively and qualitatively impairs T cell immunity and discuss potential avenues to boost the recovery of T cell compartment in host's surviving sepsis.

Sunday, December 8th

Preliminary lecture

## IMPACT OF INFECTION ON HEMATOPOIESIS AND LYMPHOCYTE HOMEOSTASIS

Dragana Janković

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Aberrant differentiation of progenitor cells in the hematopoietic system is known to severely impact host immune responsiveness. The infection-induced decrease in function of primary lymphoid organs has been postulated to be a mechanism promoting pathogen virulence. Nevertheless, whether changes in primary lymphoid organs, together with "peripheral-tolerance" in secondary lymphoid organs and tissues, contribute to immunosuppression following a pathogen challenge is poorly understood. Our recent findings revealed that systemic infectious agents, such as *T. gondii*, can induce an immunocompromised state with quantitative and qualitative deficiency in naïve T cells associated with long-term thymic atrophy. Moreover, toxoplasma infection triggers transient bone marrow hypoplasia. When accumulated during the lifetime of the host, such events, even when occurring at low magnitude, could be a contributing factor in immunological senescence.

Hematopoiesis is regulated by mesenchymal stromal cells in bone marrow. The same niche provides not only lineage-instructive differentiation signals but also supports homing of memory T lymphocytes and plasma cells. Pattern recognition receptors on mesenchymal cells have been implicated in sensing microbiota and maintaining steady-state hematopoiesis. We showed that *in vivo* administration of NOD1 ligand to germ-free mice restored the numbers of hematopoietic stem cells and precursors in bone marrow as well as serum concentrations of hematopoietic cytokines to the levels displayed by specific pathogen free control animals. Based on these findings we propose that sensing microbiota by mesenchymal cells serves as an important pathway underlying the requirement for microbiota in the maintenance of steady-state hematopoiesis.

Sunday, December 8th Session: PROTEOMICS

Invited lecture

## CYSTEINE CATHEPSINS: FROM SPECIFICITY AND SUBSTRATES TO MINIMALLY INVASIVE DIAGNOSTIC IMAGING IN CANCER

Boris Turk[1,2]

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Endolysosomal system contains over 50 hydrolases, including a number of proteases, which have a major role in numerous processes, in addition to intracellular protein turnover. Among these proteases, the most abundant are cysteine cathepsins. In human genome there are 11 cysteine cathepsins, and although structurally similar, they do not have the same roles. Several of them are also associated with MHC II-mediated antigen presentation, prohormone processing and bone resorption. In a number of inflammation-associated diseases, including cancer, arthritis and atherosclerosis, they have been found to be secreted in the extracellular milieu. Early in vitro work suggested that their primary extracellular role is the degradation of the extracellular matrix. Several of cathepsins were suggested to be involved, but the major roles seem to have cathepsins B, K, S and L. In addition, there is increasing evidence that the cathepsins are, through the cleavage of different extracellular or membrane proteins, including various CAMs, CD44, EGFR and plexins involved in the regulation of many other processes, such as regulation of Ras GTPase activity. In addition, because of their high disease-related overexpression, they were found to be excellent targets for minimally invasive diagnostic imaging, primarily in cancer. Understanding and studying their specificity and identification of their physiological substrates are therefore of major importance for understanding their signaling pathways linked with disease progression and will be further discussed, as well as development of diagnostic imaging tools targeting cathepsins.

Sunday, December 8th Session: PROTEOMICS

Inivited lecture

# NEW ALLERGENS: THEIR IMPACT ON DIAGNOSIS AND TREATMENT AND HOW TO IDENTIFY THEM.

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In modern allergology the term "allergen" is used for single allergenic molecules (proteins, glyco- and/or lipo-proteins) from an allergen source, for example, house dust mites or peanut, to name only a few. So far, extract-based *in vitro*- and *in vivo*-diagnosis was often proven to be insufficient regarding sensitivity and specificity, which is mainly due to the lack of certain allergens in diagnostic extracts. This is also relevant for treatment extracts since the lack of relevant allergens is associated with treatment failure. The identification of single allergens, their characterization and recombinant production is essential to improve allergy diagnostic tests (component-resolved diagnostics) as well as treatment options. Some single allergens are integral part of diagnostics already. In order to identify further / new allergens, proteomic methods are applied, such as SDS-PAGE with subsequent one- and two dimensional immunoblotting, several chromatography methods, N-terminal sequencing, mass spectrometry, and - depending on the allergen – peptide mass fingerprinting. In addition, the combination of these methods with peptide ligand libraries was shown to be most valuable. Allergenicity is defined by IgE-binding capacity for which sera from well-characterized patients should be used. The protein characterization is followed by epitope analysis which subsequently can be applied in microarray-based diagnostics tests. In addition, single allergens shown to be missing in the extract have already been used to spike extracts used for diagnostic tests, making the extract-based test more sensitive and specific. IgE-detection methods, be it on a protein-, be it on an epitope (peptide)-level, however, only document the patient's sensitization. They do not prove the true allergy (clinically relevant sensitization). Recently, we succeeded in optimizing the combination of molecular allergology and a cellular allergy diagnostic test that in the case of peanut allergy allows the discrimination between sensitized (asymptomatic) and allergic patients.

Sunday, December 8th Session: PROTEOMICS

Short oral presentation

## REGULATION OF mRNA EXPRESSION OF TIGHT JUNCTION PROTEINS AND PRO-ALLERGENIC CYTOKINES BY THE MAJOR KIWIFRUIT ALLERGEN ACTINIDIN *IN VIVO*

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Kiwifruit is considered as a highly nutritive fruit in the human diet, thanks to its strong antioxidant capacity and high content of vitamin C (1). Besides health-promoting properties, kiwifruit is regarded as one of the most common causes of fruit allergies, especially in the last two decades (2). Actinidin (Act d 1), a cysteine protease isolated from kiwifruit has been recognized and used as a marker allergen for sensitization to this food source (3). The gastrointestinal barrier is the first line of defense against potentially harmful antigens that normally occurred in food. Epithelial cells in the gastrointestinal tract have an important role in the maintenance of the homeostasis and actively contribute to the development of food allergy (4). Very little is known about the molecular mechanisms underlying the disintegration of the intestinal epithelial barrier by actinidin. The aim of this study was to explore the effects of kiwifruit allergen Act d 1 on intestinal permeability, tight junction (TJ) proteins as well as pro-allergenic cytokines gene expression in the different parts of mice intestine (proximal, distal and colon). Orally administered Act d 1 increased intestinal permeability. This effect was accompanied by a change in the mRNA expression pattern of TJ proteins and innate pro-allergenic cytokines IL-25, IL-33 and thymic stromal lymphopoietin (TSLP) compared to the control. Act d 1, based on its proteolytic activity, affects mRNA expression of tight junction proteins and pro-allergenic cytokines *in vivo*. The obtained results connect the proteolytic activity of Act d 1 protease with epithelial barrier dysregulation and shed new light on the understanding of the sensitization process.

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Sunday, December 8th Session: PROTEOMICS

Short oral presentation

## NKG2D AS A MARKER OF ACTIVE TUMOR-ANTIGEN SPECIFIC CD8$^+$ T CELLS DURING ANTITUMOR IMMUNE RESPONSE IN MOUSE MELANOMA MODEL

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Despite of its clinical success, majority of cancer patients remain unresponsive to currently available checkpoint inhibitors, leaving the quest for finding new modulators of tumor-specific immunity open. To understand the time-scale position of specific immune response during tumor control, we established a bioluminescence imaging model to monitor the interplay between immunogenic tumor and tumor-antigen specific immunity. By using B16-OVA-Luc2 cells, we monitored the timeframe of tumor immune control and escape in the mice immunized with a model tumor antigen ovalbumin (OVA-mice). The antitumor immune response in OVA-mice was found to be critically dependent on IFN-γ and CD8$^+$ T cells and the tumor escape was strongly correlated with reduced clonality and loss of proliferative capacity of OVA-specific CD8$^+$ T cells within tumor microenvironment. Such tumor-antigen specific T cell dysfunction was not associated with the expression of exhaustion / dysfunction markers such as PD-1, LAG3, TIM3 and KLRG-1. Instead, we found that the reduction of cell surface expression of NKG2D on OVA-specific CD8$^+$ T cells, together with the altered transcription factor expression that control T cell differentiation and function, are coinciding with the tumor escape from immune control. Our findings suggest NKG2D as a marker of active tumor antigen-specific CD8$^+$ T cells and potentially involved in controlling their functionality.

Sunday, December 8th Session: AUTOIMMUNITY

Invited lecture

# T CELLS IN TYPE 1 DIABETES: ROLE AND POSSIBILITIES OF MODULATION

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Type 1 diabetes (T1D) is T-cell dependent immune-mediated disease, in which the insulin-producing pancreatic beta cells are destroyed. T1D progresses through asymptomatic stages characterized by the appearance of autoantibodies, then dysglycemia and finally hyperglycemia and overt disease. These immunological and metabolic features can identify persons at high risk for T1D. Disease development reflects a failure of immune tolerance mechanisms that control autoreactive T cells with proinflammatory cytokine profiles. In that context, both CD4 + helper and CD8 + cytotoxic T cells exert important role in T1D development. Thus, CD4+ T cells are required for activation of CD8+ T cells, then activated effector T cells infiltrate the pancreas and while CD4+T cells contribute to beta cell death through macrophage activation, CD8+ T cells directly kill beta cells in an antigen-dependent manner. When immunotherapy of T1D is concerned, previous studies suggested that anti-CD3 monoclonal antibodies (mAbs) may affect immune responses by induction of T regulatory cells (Tregs), the release of inhibitory cytokines, or depleting effector T cells. Treatment with anti-CD3 mAbs in patients with recent-onset T1D reduces the loss of beta-cell function, even as long as 7 years after diagnosis. Moreover, modulation of T cell function by anti-CD3 mAbs, delays the progression to T1D for 2 years in subjects at high risk for T1D. In that context, high-risk individuals who are progressing to T1D more slowly have increased frequency of CD8+ T cells, and express markers of T cell exhaustion and nonresponsiveness, TIGIT and KLRG1. These findings have directed novel therapeutic approaches in T1D and high risk individuals to the resetting the balance between Tregs and T effector cells.

Invited lecture

## NEW APPROACHES FOR SELECTIVE IMMUNOTHERAPY OF AUTOIMMUNE DISEASES BY ENGINEERED CHIMERIC MOLECULES

Nikolina Mihaylova[1], Kalina Nikolova-Ganeva[1], Iliyan Manoylov[1], Gabriela Boneva[1], Silvya Bradyanova[1], Melinda Herbáth[2], József Prechl[2], Andrey Tchorbanov[1]
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Aim: Self-specific B and T lymphocytes play main roles in pathogenesis of autoimmunity and their selective elimination is a legitimate goal in the efforts to control disease progression. The complexity of autoimmunity is characterized by autoreactive T cell activation and the generation of IgG auto-antibodies specific to self-antigens - dsDNA, histones, nucleosomes, GAD65. Inhibitory B cell surface co-receptors such as FcγRIIb, CR1 and CD22 negatively regulate BCR signaling and engagement of these molecules inhibit B cell activation. One of the mechanisms for specific B cell suppression is the cross-linking of the surface immunoglobulins with the inhibitory receptors by IgG-peptide-containing immune complexes. We hypothesize that it may be possible to down-modulate the activity of autoreactive T and B cells in lupus- or diabetes-prone mice as well as in humanized NSG lupus and diabetes mouse models by treating them with protein engineered molecules, which co-crosslink the BCR and inhibitory receptors. Methods: Protein chimeric molecules construction; MRL, NOD and humanized Immunodeficient NSG mouse models. The suppressive activities of the engineered molecules were tested *in vitro* and *in vivo.* The levels of auto-antibodies and cytokines in the mice sera as well as the apoptosis, the number of IgG-producing plasma cells and kidney injuries were quantified by ELISA, FACS, ELISpot, protein array and Histology. Results: By protein engineering we constructed several chimeric molecules by coupling different self-mimicking peptides to an anti-mouse FcγRIIb or anti-human CR1-binding monoclonal antibody. The administration of these chimeric molecules to autoimmune-prone or to humanized NSG mice, transferred with PBMC from autoimmune patients resulted in the reduction of the levels of IgG auto-antibodies, of the albumin and glucose serum levels, and in prevention of the glomerulonephritis development. Conclusion: Engineered chimeric molecules for targeting of antigens to the inhibitory receptors on self-reactive B cells are a tool for directed immune response modulation.

Sunday, December 8th Session: AUTOIMMUNITY

Short oral presentation

## *Trichinella spiralis* PRODUCTS, POWERFUL MODULATORS OF AUTOIMMUNITY

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More than a decade ago we have made our first contribution to Hygiene hypothesis by discovering that infection with *Trichinella spiralis* could dampen or even prevent the development of experimental autoimmune encephalomyelitis (EAE), an animal model of human autoimmune disease multiple sclerosis. *T. spiralis* exerts its impact on the host immune system through excretory-secretory muscle larvae (ES L1) products. Among the mechanisms underlying observed immunomodulation, anti-inflammatory/regulatory mechanisms are the most significant. The application of *T. spiralis* ES L1 products through intraperitoneal injection or via tolerogenic dendritic cells (ES L1 TolDCs) proved to be successful in EAE amelioration, but some limitations regarding their possible application in humans were imposed. In aim to create novel therapeutic approach for multiple sclerosis, the new delivery system for ES L1 products was designed, made of biodegradable nano-fibers. This allowed usage of much smaller amounts of ES L1 during application (compared to above mentioned paths). The subcutaneous implantation procedure for ES L1-nanocarrier implies performance of a small surgical intervention ones before the induction of relapse/remitting form of EAE in DA rats. The results obtained on the systemic as well as on the target tissue level indicated a substantial potential of treatment with ES L1-nanocarrier to restrain disease development and progression in animal model, and provided insight in mechanisms crucial for soothing inflammation and restoring immune homeostasis. (Grant No: 173047, 175102).

Short oral presentation

## ATRA- AND TGF-β-LOADED MICROPARTICLES AMELIORATE TYPE 1 DIABETES IN MICE

Ivan Koprivica[1], Dragica Gajić[1], Tamara Saksida[1], Eugenio Cavalli[2], Dominick Auci[3], Sanja Despotović[4], Nada Pejnović[1], Stanislava Stošić-Grujičić[1], Ferdinando Nicoletti[5], Ivana Stojanović[1]

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Type 1 diabetes (T1D) is an autoimmune disease in which a strong inflammatory response causes the death of pancreatic β-cells. Attempts to induce anti-inflammatory/regulatory immune mechanisms that would attenuate disease progression have shown little or no beneficial effects. We introduced microparticles (MPs) loaded with Transforming Growth Factor β (TGF-β) and All-Trans Retinoic Acid (ATRA), both known stimulators of T regulatory cell (Treg) differentiation and stabilization. Male C57BL/6 mice were treated with multiple low doses of streptozotocin to induce T1D, and orally treated with vehicle, empty MPs, or ATRA- and TGF-β-loaded MPs for 10 days (every other day). T1D incidence and immune cell infiltration into the pancreatic islets were lower in ATRA/TGF-β-MPs-treated mice. In Peyer's patches (PP), ATRA/TGF-β MPs up-regulated tolerogenic dendritic cells (tolDC). Additionally, IL-1β expression was reduced in PP, as was the ratio of iNOS/Arginase expression, reflecting a less inflammatory environment. This was accompanied by reduced proportion of Th1 and Th17 cells and up-regulation of Treg. IL-17 expression within $CD4^+$ T cells from PP was also lower and was accompanied by down-regulation in the expression of RORγt, a key transcription factor of IL-17. In the pancreatic lymph nodes (PLN), the situation was similar to PP regarding the down-regulation of Th1 cells. Additionally, in response to ATRA/TGF-β MPs treatment, the proliferation of T effector cells was reduced in PLN, while Treg proliferated more. The presence of $CTLA\text{-}4^+PD1^+$ and $CD39^+IL\text{-}10^+$ Treg populations was also increased, indicating higher suppressive activity. In conclusion, ATRA and TGF-β released from MPs successfully ameliorated T1D by potentiating tolDC and Treg and inhibition of Th1 cell differentiation in gut-associated lymphoid tissue and the draining lymph nodes, thus blocking the entrance of immune cells into the pancreatic islets and protecting β-cells from further destruction.

# Sunday, December 8th Session: AUTOIMMUNITY

## Short oral presentation

### EXOGENOUS IL-33 PREVENTS MLD-STZ INDUCTION OF DIABETES AND ATTENUATE INSULITIS IN PREDIABETIC NOD MICE

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Background and aims: Type 1 diabetes is an autoimmune disease caused by the immune-mediated destruction of pancreatic β-cells. Prevention of type 1 diabetes requires early intervention in the autoimmune process. $CD4^+Foxp3^+$ Tregs participate as one of the most important cell types in limiting the autoimmune process. We have previously shown that IL-33R (ST2) deletion enhanced susceptibility to multiple low dose streptozotocin (MLD-STZ) induced diabetes. Aim: The aim of this study was to investigate the preventive and therapeutic effect of IL-33 in MLD-STZ induced diabetes and to delineate the mechanisms of its influence on autoimmune attack. Material and metods: For the induction of diabetes C57BL/6 mice were treated with five doses of 40 mg/kg STZ, and 0.4μg rIL-33 was administrated per mouse, four times, every second day from the day of disease induction. 16 weeks old NOD mice were treated with 6 injections of 0,4μg/mouse IL-33 (every second day). Glycemia, glycosuria and HbA1c levels were measured after diabetes induction and histological and immunohistochemical parametars in pancreatic islets were evaluated on day 28. Cellular make up of the pancreatic lymph nodes and islets were evaluated by flow cytometry. Results: IL-33 given simultaneously with the application of STZ completely prevented the development of hyperglycemia, glycosuria and attenuated islet mononuclear cells infiltration. IL-33 treatment enhanced the bias toward Th2 immune response and increased the frequency and number of $ST2^+$ Tregs. This was accompanied by higher number of IL-13 and IL-5 producing $CD4^+$ T cells and increased presence of $ST2^+Foxp3^+$ Tregs in pancreatic lymph nodes and islets. Using IL-33 also promotes islet infiltration with M2 macrophages. IL-33 given 6 and 12 days after diabetes induction partially attenuates clinical signs and influx of inflammatory cells in the islets and had therapeutic effect. Conclusion: We provide evidence that exogenous IL-33 completely prevents the development of T cell mediated inflammation in pancreatic islets and consecutive development of diabetes in C57BL/6 mice by facilitating the induction Treg cells. To extend this finding for possible relevance in spontaneous diabetes, we showed that IL-33 attenuate insulitis in prediabetic NOD mice. This work was supported by a grant from the Serbian Ministry of Science and Education (projects no.175069 and 175071), research grant from University of Kragujevac, Faculty of Medical Sciences, Serbia (project no. JP 12/17) to SP and grant from Swiss National Science Foundation (project no. IZ73Z0_152407) to ML.

Poster presentation

## THE DECREASE OF TOLEROGENIC ILC3 AND TREG CELLS IN SMALL INTESTINE CORRELATES WITH THE PROGRESSION OF TYPE 1 DIABETES IN MICE

Bojan Jevtic[1], Tamara Saksida[1], Dragica Gajic[1], Ivan Koprivica[1], Verica Paunovic[2], Ivana Stojanovic[1], Nada Pejnovic[1]

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Type 1 diabetes (T1D) is a chronic autoimmune disease characterized by the imbalance between the CD4 or CD8 T effector (Teff) cells and the FoxP3$^+$CD4 T regulatory cells (Tregs) that leads to pancreatic beta-cells destruction causing insulin deficiency. Environmental factors, diet and microbiome are associated with the recent rise in T1D incidence. Intestinal immune cells must maintain a tolerogenic response in the gut that involves the development of Tregs. Recent data show that IL-2-producing type 3 innate lymphoid cells ILC3s (IL-2$^+$ILC3) in the small intestine are essential for maintaining FoxP3$^+$ Tregs and oral tolerance to dietary antigens and reveal the previously unknown direct communication between ILC3s and Treg cells in the gut. We investigated the frequencies of small intestine lamina propria IL-2$^+$ILC3s and FoxP3$^+$ Tregs during transition from prediabetes to diabetes in young and old female NOD mice. 20 weeks old, diabetic NOD mice had higher frequencies of Lin$^{neg}$CD45$^+$RORγt$^+$CD127$^+$ ILC3s in small intestine lamina propria compared to 4 weeks of age-young NOD mice. However, the frequencies of IL-2-producing ILC3s and CD4$^+$CD25$^{hi}$FoxP3$^+$ Tregs were significantly lower in diabetic NOD mice compared to young, prediabetic mice. We next investigated how microbiota change before diabetes induction is reflected on Treg and ILC3 populations. Male C57BL/6 mice were treated with broad spectrum antibiotics (ABX) for 14 days and then T1D was induced by multiple low doses of streptozotocin (STZ). *Ex vivo* cell analyses was done on day 10 after the first STZ injection. The significantly higher incidence of T1D observed in ABX-treated mice correlated with significantly lower frequencies of IL-2-producing ILC3s and FoxP3$^+$Tregs in small intestine lamina propria compared to mice treated with STZ only. The obtained findings show that the decrease of tolerogenic ILC3s and FoxP3$^+$Tregs in small intestine is associated with the progression and higher incidence of T1D.

## Sunday, December 8th Session: AUTOIMMUNITY

### Poster presentation

### REPRODUCTIVE MANIFESTATIONS OF AUTOIMMUNITY IN MOUSE MODEL OF SYSTEMIC LUPUS ERYTHEMATOSUS

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Introduction: Systemic Lupus Erythematosus (SLE) is an autoimmune systemic disease characterized by the appearance of autoantibodies directed against nuclear, cytoplasmic and cell- surface antigens. The fact that women are predisposed to SLE and the symptoms are exacerbated during active reproductive years and pregnancy emphasizes the importance of female hormones in the progression of autoimmunity. Hence, on account of the common reproductive complications, it is urgent to address the question how SLE can influence female fertility and vice versa. Mouse models of SLE are suitable tools for studying in details the interactions of different systems in the context of the present disease. Purpose: To follow the effect of SLE manifestations on the production and development of mouse oocytes. Method: Lupus-like symptoms were induced through intraperitoneal injection of hydrocarbon oil pristane in non-autoimmune Balb/C mice. The experimental animals were characterized using flow cytometry, ELISpot and ELISA. The collected oocytes were analyzed based on chromatin, tubulin and actin structures using Hoechst 33258, FITC-labeled alpha-tubulin antibody and rhodamine-labeled phalloidin, respectively. Results: A single i.p. injection of pristane led to the production of different autoantibodies accompanied by massive glomerular depositions of IgG-containing immune complexes in the kidneys, and proteinuria. The total number of obtained metaphase oocytes from lupus mice was significantly lower compared to healthy controls. The maturation rate, i.e. the proportion of eggs reaching metaphase II, was also lower for lupus mice compared to control animals. In addition, oocytes from lupus mice presented specific abnormalities, including long chromosomes, disorganized spindle and missing actin cap. Discussion: Pristane-induced mouse model of lupus exhibited impairments of the reproductive system which may result due to disease activity, autoantibodies or damage in molecular mechanisms through the process of reproduction. The model will be further developed and the direct effect of female hormones on disease outcome will be studied in details.

Poster presentation

# IgG GLYCOSYLATION IN ACHR-AB AND MUSK-AB MYASTHENIA GRAVIS IDENTIFIED BY LECTIN AFFINITY ELECTROPHORESIS

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*Myasthenia gravis* (MG) is a disease with disorder of transmission at the neuromuscular junction, characterized by weakness and fatigability of the skeletal muscle. The most frequent is acquired autoimmune form, with acetylcholine receptor autoantibodies (AchRAb) or muscle-specific tyrosine kinase autoantibodies (MuSKAb). Measurements of antibodies enable greater certainity and speed during the diagnostic procedure. The carbohydrate moiety of human serum IgG shows high structural multiplicity, particularly associated with various inflamatory, malignant and autoimmune diseases. However, there are not much literature data about IgG glycosylation in MG, and no one based on lectin interaction. The aim of the present work was examination of IgG glycosylation in sera of patients with two type of myastenia (AchRAb and MuSKAb positive) using lectin affinity electrophoresis with mannose-specific lectin ConA in the first dimension and monospecific anti-human IgG antibodies in second dimension. Control sera were tested in the same way. AchRAb and MuSKAb were determined using radioimmunoassay (RIA), and positive sera were further examined. Two-dimensional electrophoresis of MG sera without lectin resulted in the pattern very similar to controls. This was not the case with sera from RA patients whith massive IgG precipitate or myeloma sera with characteristic monoclonal fraction, obtained in our previous research. However, lectin ConA in the first dimension made difference, binding IgG in MG sera with higher affinity (determined by retardation coeficient) compared to control sera. Those results indicated better exposure of the three-mannose core, due to fewer terminal galactose residues in IgG oligosaccharide chains in MG, compared to control.

Our results are in accordance with some literature data obtained by mass spectrometry. More data (the level of fucose or bisecting N-acetylglucosamine) will be obtained using other lectins. Such research can be significant because N-glycosylation of the IgG Fc moiety influences its biological activity by modulating the interaction with Fc receptors.

Poster presentation

## VARIANT C.454-397T>C OF ESTROGEN RECEPTOR A INFLUENCES RESULTS OF LUPUS ANTICOAGULANT SCREENING AND FIBRINOGEN MEASURMENT IN PATIENTS WITH ACUTE MYOCARDIAL INAFARCTION - A PILOT STUDY

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Estrogens are steroid hormones which, beside reproductive organs, influence other physiological systems i.e. cardiovascular system, skeleton, cognition, etc. Their effects are achieved via estrogen receptors (ER) - α and β - both being polymorphic. Previously, a possible association was assessed between variants of ERα gene and antiphospholipid syndrome (APS), an autoimmune condition, predisposing to acute myocardial infarction (AMI). Lupus anticoagulant (LA) are one of the most frequently found antibodies in APS and fibrinogen level can be considered as an indicator of inflammation accompanying AMI. Our objective was to examine the influence of ERα c.454-397T>C variant on results of LA screening and fibrinogen measurement in patients with AMI. Study involved 120 patients (54 males and 66 females) with AMI. Commercially available tests were employed for LA screening and fibrinogen measurement. ERα genotyping performed using PCR-RFLP analysis. Results were analyzed using Student's t-test. On average, higher results of LA1 test were encountered in patients who were homozygote for Erα c. 454-397C allele (46.4±16.07 s vs 39.9±6.41 s; P=0.008). LA2 results in patients with Erα c. 454-397CC genotype (51.8±13.82 s) were significantly above (P=0.018) values in patients with other genotypes (39.9±6.43). Higher fibrinogen concentrations were measured in carriers of Erα c. 454-397C allele (5.4±1.34 g/L vs 6.3±1.90 g/L; P=0.007). Also, homozygosity for Erα c. 454-397C allele was associated with difference in certain demographic characteristics i.e. higher body mass (82.7±16.38 kg vs 74.8±14.75 kg; P= 0.030) and height (175±7.8 cm vs 168±9.4 cm; P=0.002). Gender related differences were not present for either of the reported parameters. Our pilot results indicate higher values of LA screening tests and fibrinogen measurement in the presence of the ERα c. 454-397C allele. Further studies are necessary to comprehensively evaluate the causes and significance of these associations.

Poster presentation

## SEXUAL DIMORPHISM IN THE SEVERITY OF RAT COLLAGEN-INDUCED ARTHRITIS: THE RELEVANCE OF T FOLLICULAR CELL HELP TO B CELLS

Mirjana Dimitrijević[1], Nevena Arsenović-Ranin[2], Duško Kosec[3], Biljana Bufan[2], Mirjana Nacka-Aleksić[4], Ivan Pilipović[3], Gordana Leposavić[4]

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Collagen-induced arthritis (CIA) is a well-established experimental model mimicking many immunopathogenic and clinical aspects of rheumatoid arthritis (RA), including sexual dimorphism in the clinical presentation. Our previous study showed that a more severe disease in female compared with male rats correlated with more robust Th17 response reflecting sexual dimorphism in Th17/Treg axis plasticity. Given that autoantibodies play a significant role in the immunopathogenesis of RA and CIA, in the present study the germinal center (GC) reaction in the lymph nodes draining inflamed joints and adjacent tissue (dLNs) was examined for putative sexual dimorphism. Female rats mounted greater serum collagen II-specific IgG response than their male counterparts. This dimorphism correlated with the higher frequency of GC B cells in female compared with male dLNs. Consistently, the frequency of activated/proliferating Ki67+ cells among dLN B cells was higher in females than in males. This was associated with the shift in dLN T follicular regulatory (Tfr)/T follicular helper (Tfh) cell ratio towards Tfh cells in females, and greater densities of CD40L and CD40 on their dLN T and B cells, respectively. The higher Tfh cell frequency in females was consistent with the greater dLN expression of mRNA for IL-21/27, the key cytokines involved in Tfh cell generation and help to B cells. Additionally, in collagen II-stimulated female rat dLN cell cultures, IFN-γ/IL-4 ratio was shifted towards IFN-γ. Consistently, serum ratio between pathogenic IgG2a and protective IgG1 collagen II-specific antibodies was shifted towards the former in females. Thus, the study suggests that targeting T/B cell interactions should be considered in further translation research aimed to design sex-specific therapies for RA.
(This work was supported by the grant 175050 from MPNTR RS).

Sunday, December 8th Session: AUTOIMMUNITY

Poster presentation

## AUTOANTIBODIES IN RA PATIENTS BEFORE AND AFTER METHOTREXAT TREATMENT

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Rheumatoid arthritis (RA) with a frequency of 0.5% to 1% in the general population is the most common systemic autoimmune disease associated with chronic inflammation and joint destruction. Sometimes it is difficult to differentiate RA from other forms of arthritis in the onset of the disease, so early diagnosis can be quite difficult. Autoantibodies found in RA can be present and detected years before the development of clinical symptoms. Due to toxic drugs that can cause very dangerous side effects, a highly specific marker is needed to identify patients with RA before joint damage occurs. The most significant feature of RA is the presence of specific antibodies. The IgM Rheumatoid factor (RF) is detected with a sensitivity of 60-70% and a specificity of 80-90%. However, antibodies to cyclic citrulline peptide (CCP antibodies) have a higher specificity of up to 98%, and similar sensitivity of 68-80%. Anti-CCP antibodies are a potentially significant marker for the diagnosis and prognosis of RA because they are: sensitive and specific more than IgM RF in both early disease development and fully developed clinical features, they can predict eventual development in RA from undifferentiated arthritis, they are marker of disease erosivity, they can be detected in healthy people years before the clinical development of RA. The aim of this study is to compare the results of anti-CCP antibodies in patients with differentially diagnosed RA, with the results of anti-CCP antibodies after Methotrexate therapy.This study enrolled 30 patients with the ACR criteria for RA, who had elevated anti-CCP antibodies at the outset of the disease. After Metotrexat therapy no significant changes of anti-CCP levels were evaluated. We detected that anti-CCP antibodies were positive in 90% of RA patients at the early onset of the disease and after Metotrexat therapy we measured approximately the same value.

Sunday, December 8th Session: AUTOIMMUNITY

Poster presentation

## SALIVARY PROTEOMIC ANDCEA ANALYSIS IN HEALTHY AND SJÖGREN'S SYNDROME PATIENTS

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Sjögren's syndrome (SS) is a complex autoimmune disease, characterized by a progressive hypofunction and inflammation of salivary and lachrymal glands. Diagnosis of SS is problematic, because it often relies on nonspecific signs and symptoms with no specific biomarker(s). Since saliva directly reflects salivary gland inflammation and damage, this body fluid appeared as useful tool for new biomarker research. In the last few years, salivary proteomic studies were conducted providing considerable contribution in the search of SS biomarkers. As a body fluid, saliva contain a number of glycosylated proteins, including heavily glycosylated carcinoembryonic antigen (CEA), decsribed as inflammatory protein. This study aimed to investigate salivary proteome and CEA as a potential salivary biomarker in Sjogren's syndrome patients. Quantitative salivary research was conducted on saliva samples collected from healthy women and SS female patients. The protein concentration was determined by using BCA protein assay kit (ThermoScientific), while proteome was analysed using electrophoretic techniques. The levels of CEA were measured using immunoradiometric assay IRMA CEA (INEP).The results showed altered salivary proteome of SS pateints compared to healthy subjects, especially in the area of smaller molecular masses. Beyond the proteome, CEA analysis showed that SS is associated with significantly increased CEA level in SS patients. The obtained results indicate that salivary CEA could be a potentially useful diagnostic and follow-up SS biomarker. Even more, due to many indicated roles of CEA family members, CEA presence could be functionally relevant in the pathogenesis of disease.

# Sunday, December 8th Session: EPIGENETICS

## Invited lecture

## THE PAST, PRESENCE AND FUTURE OF TARGETING EPIGENETIC MARKS: FROM CHEMICALS AND DNA VIA PROTEINS TO RNA AND CLINICAL GENE THERAPY

Marianne G. Rots

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The ability to target DNA specifically at any given position within the genome allows many intriguing possibilities and has inspired scientists for decades. Early gene targeting approaches exploited chemicals or DNA oligonucleotides to interfere with the DNA at a given locus in order to inactivate a gene or correct mutations. In addition to the promise of gene correction, scientists soon realized that genes could be silenced and even re-activated without inducing potentially harmful DNA damage, by targeting transcriptional modulators to a particular gene.[1] These first generation programmable DNA binding domains (polyamides, triplex forming oligonucleotides), however, proved difficult to fuse protein effector domains to.[2] The engineering of gene-targeting proteins (ZFPs, TALEs) circumvented this problem, but required new protein fusions for each locus. The more recent introduction of CRISPR/Cas offers a flexible approach to target fusion dCas-proteins to the locus of interest using cheap designer RNA molecules. Many research groups now exploit this platform and the first human clinical trials have been initiated: CRISPR/Cas has kicked off a new era of gene targeting and is revolutionizing biomedical sciences. Following the increasing awareness of frequent epigenetic dysregulations in human diseases, and the success of clinically approved epigenetic drugs, the gene targeting platforms have been repurposed to edit epigenetic signatures at any given locus.[3] Currently, this approach has convincingly demonstrated causality of various epigenetic modifications in instructing gene expression and has provided exciting indications supporting the promise to interfere with disease-associated gene expression dysregulations. Some reports indicate the feasibility of long-term gene repression as well as re-expression,[3] but solid guidelines on how to stably interfere with epigenetic gene regulation in any given chromatin environment are largely lacking. The expected progress in this aspect, together with developments in safe delivery methods, open novel therapeutic avenues and editing epigenetic signatures might realize the "*curable epigenome*" concept for currently untreatable diseases. Financial support includes an EU SNN grant and an H2020 ITN grant (www.EpiPredict.eu). Networking activities are supported by EU COST CM1406 (www.EpiChemBio.eu)

Sunday, December 8th Session: EPIGENETICS

Invited lecture

# ERASING EPIGENETIC MEMORY: PRONOUNCED SEQUENCE PREFERENCE OF MAMMALIAN TET ENZYMES GUIDES ACTIVE DNA DEMETHYLATION

Tomasz P Jurkowski

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Epigenetic memory in the form of cytosine methylation is essential for vertebrate development and the formation of cellular identity (Lee, Hore and Reik, 2014). Active removal of DNA methylation by the action of the TET hydroxylase family is crucial for assisting cells to gain developmental potency, both *in vivo* and in culture, through the creation of induced pluripotent stem cells (Costa et al., 2014; Hore et al., 2016). Despite this, little is known about the molecular mechanisms that target the TET proteins to their site of action, and currently, it is assumed that all sites demethylate with equal efficiency. We report that mammalian TET enzymes show strong preference (>500 fold) for oxidation of a subset of CG containing hexamers (*in vitro* and in cultured cells). These preferred sequences constitute binding sites for developmental transcription factors whose DNA binding activity is regulated by DNA methylation. X-ray structural analysis and molecular dynamics simulations suggest that TET use indirect readout to sense the sequence context flanking CG sites. These results are significant for understanding epigenetic reprogramming during development and have implications for synthetic biology and epigenetic editing.

Short oral presentation

## VARIANT CCG REPEATS WITHIN *DMPK* EXPANSIONS AND SURROUNDING CpG SITES ARE HETEROGENEOUSLY METHYLATED IN BLOOD CELLS OF MYOTONIC DYSTROPHY TYPE 1 PATIENTS

Jovan Pešović[1], Stojan Perić[2,3], Miloš Brkušanin[1], Goran Brajušković[1], Vidosava Rakočević-Stojanović[2,3] & Dušanka Savić-Pavićević[1]

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Myotonic dystrophy type 1 (DM1) is phenotypically one of the most variable monogenic diseases. It is caused by an expansion of CTG repeats in the *DMPK* gene. The number of CTG repeats is the main factor influencing disease severity. However, the effect of the causing mutation can be modified by different factors. An example of genetic factors are variant repeats (CCG, CTC, GGC or CAG) scattered among CTG repeats and found in about 5% of DM1 patients. We previously showed that variant repeats delay age at onset in DM1 patients by stabilizing *DMPK* expansions in somatic cells. Since our patients had various patterns of CCG repeats, we assumed that CCG repeats might be methylated themselves, similarly to CGG and G4C2 repeats in the expansions associated with Fragile X syndrome and Amyotrophic lateral sclerosis/Frontotemporal dementia, respectively. Therefore, our aim was to investigate whether CCG variant repeats in *DMPK* expansions were associated with changes in epigenetic marks both in the repeat region itself and in the surrounding CpG sites within a large CpG island, in which the *DMPK* repeat region is embedded. By using two originally designed methods: methyl-specific RP-PCR on a bisulfite-converted genomic DNA and classical RP-PCR on genomic DNA digested by *Ssi*I enzyme. We discovered that variant CCG repeats were heterogeneously methylated within *DMPK* expansions. Targeted bisulfite sequencing revealed heterogenic methylation of CpG islands upstream and downstream of repeat tract. Based on the observation that the relative degree of methylation of CCG repeats and CpG islands depends on quantity and patterns of CCG repeats, it is hypothesized that methylation is initiated on CCG repeats and spreads locally on the surrounding CpG islands. The discovery of methylation of CCG repeats in *DMPK* expansions opens questions about the role of epigenetic mechanisms in the stabilization of *DMPK* locus.

## Sunday, December 8th Session: EPIGENETICS

Short oral presentation

### THE IMPORTANCE OF STUDYING miRNAs IN ORAL CANCER

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Oral cancer arises in oral cavity and is the most common type of head and neck cancers. Epidemiology of oral cancer is worrisome, due to increased incidence worldwide, especially among younger people. Exposure to risk factors, such as smoking, alcohol consumption and infection with human papilloma virus, significantly increase risk to oral cancer susceptibility. In addition, accumulation of genetic and epigenetic changes leads to carcinogenesis of oral epithelial cells. The first line of oral cancer treatment is surgical resection. Despite successful removal of cancerous tissue, approximately 20% of patients will develop recurrence. As oral cancer is still challenging contemporary medical practice and patients' outcome, revealing molecular complexity of this cancer type is needed. That is why scientific efforts are put into identification of more sensitive and novel molecular biomarkers for discrimination of oral cancer and surrounding non-cancerous tissue as well as prognostic biomarkers.

A class of small non coding RNA molecules, micro RNAs (miRNAs) is nominated as potential molecular biomarkers due to its stability in tissues. Using methodology of high-throughput platforms, numerous studies identified deregulated miRNAs in oral cancer compared to adjacent non-cancerous tissue, but obtained data are highly inconsistent. In order to find the most commonly deregulated miRNAs characteristic for oral cancer, we conducted the meta-analysis of previously published data. The list consisted of twenty commonly deregulated miRNAs in oral cancer compared to adjacent non-cancerous tissue. The biological meaning of miRNAs meta-list in oral cancer was characterized by bioinformatics enrichment analysis. Validation of identified miRNAs in an independent set of oral cancer clinical specimens by more sensitive methods, such as quantitative Real Time PCR is warranted. Based on our obtained results so far, it will be illustrated why it is important to continue studying miRNAs in oral cancer.

Sunday, December 8th Session: EPIGENETICS

Short oral presentation

# APPLICATION OF EPIGENOMICS FOR THE STUDY OF TUMOR HETEROGENEITY IN NON-SMALL-CELL LUNG CANCER

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Tumors evolve through acquisition of genetic and epigenetic alterations that lead to diversification of tumor subclonal populations. One consequence of clonal evolution is phenotypic tumor heterogeneity manifested by spatial and temporal differences in gene and surface marker expression, aggressiveness, and response to therapy. Genetic mutations may activate oncogenes or abrogate function of tumor suppressor genes, but also give rise to modified proteins - neoantigens that can be recognized by the immune system to trigger adaptive response. Yet, cancer cells have evolved several mechanisms to avoid recognition by the immune cells or halt their response. Similarly, human tumors contain large numbers of epigenetic changes affecting gene expression and chromosomal stability. However, the extent of epigenetic alterations, both between and within spatially separated tumour regions, and their impact on tumor evolution is not yet understood. DNA methylation is one of the most studied epigenetic marks, frequently deregulated in cancer. Here we will show an application of reduced-representation bisulfite sequencing (RRBS) for the study of DNA methylation on a cohort of 36 non-small cell lung cancer (NSCLC) patients. Leveraging clinically and multi-omics fully annotated multiregional cohort of NSCLC patients from the TRACERx trial, we have previously identified a novel epigenetic mechanism underlying immune evasion in cancer, through DNA methylation mediated silencing of neoantigens. Here we use new algorithms to deconvolute pure tumor methylomes and evaluate the degree of both inter- and intra-tumour epigenetic heterogeneity to inform on the relationship between genetic and epigenetic tumour evolution, tumor phenotype and clinical outcomes.

Sunday, December 8th Session: EPIGENETICS
Short oral presentation

# GENE METHYLATION IN HEAD AND NECK TUMORS

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Neoplastic transformation of human cells is in part attributed to a summation of genetic and epigenetic modifications. Most commonly, epigenetic control of gene expression is achieved via DNA methylation, histone modification and/or regulation by small non-coding RNAs. Tumor suppressor gene (TSG) promoter hypermethylation represents a powerful mechanism of transcription silencing and the INK4a-ARF locus, encoding two tumor suppressors, p16INK4a and p14ARF, is one of the alteration hotspots in human neoplasms.

Our research group has determined the methylation status of p14ARF and p16INK4a in a series of head and neck tumors. Benign (pleomorphic adenomas) and malignant salivary gland tumors (carcinoma ex pleomorphic adenomas and mucoepidermoid carcinomas), as well as oral squamous cell carcinomas (OSCC) have been analyzed using methylation specific PCR. Hypermethylation of p16INK4a and p14ARF appears to be a quite frequent event in all investigated pathologies; namely, the frequency ranged from 60 up to 100 %, depending on tumor type. The most remarkable finding was that all of the analyzed mucoepidermoid carcinoma samples exhibited p14ARF promoter hypermethylation. On the other hand, only 20% of the analyzed normal salivary glands exhibited this epigenetic modification. Interestingly, it seems that p16INK4a hypermethylation and p14ARF hypermethylation are unrelated events. In OSCC patients three types of samples were analyzed (tumors, tumor-free surgical margins and unaffected oral mucosa) for the simultaneous occurrence of these alterations and p14ARF was methylated in all three samples from 62% of patients, and p16INK4a in 35% of patients.

p14ARF and p16INK4a promoter hypermethylation do not appear to be significantly related to tumor phenotype, i.e. to clinicopathological parameters in any of the examined neoplasms. However, their high frequency points to the importance of this epigenetic event in head and neck carcinogenesis and should be further analyzed in a larger cohort.

Poster presentation

## THE METHYLATION STATUS OF MGMT IN SERBIAN PATIENTS WITH DIFFUSE GLIOMA

Nikola Jovanović[1], Tatjana Mitrović[1], Vladimir J. Cvetković[1], Vesna Nikolov[2], Svetlana Tošić[1], Jelena Vitorović[1], Slaviša Stamenković[1], Aleksandar Kostić[2], Nataša Vidović[3], Miljan Krstić[3], Tatjana Jevtović-Stoimenov[4], Dušica Pavlović[4]

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Epigenetic alterations represent one of the key mechanisms which drive development of diffuse gliomas. Among various alterations that occur as a result of disruption of DNA methylation patterns during diffuse glioma carcinogenesis, the methylation of the promoter of the $O^6$-methylguanine-DNA methyltransferase (*MGMT*) gene was found to be prognostic and predictive epigenetic biomarker with a recognized clinical value. MGMT is DNA-repair enzyme which induces chemoresistance due to its ability to counteract with cytotoxic effect of alkylating agents such as Temozolomide (TMZ). Epigenetic silencing of *MGMT* through methylation of cytidine-phosphate-guanosine dinucleotides (CpG) in promoter region can lead to a more favorable outcome and treatment response. The goal of our study was to screen Serbian diffuse glioma patients for a methylation status of *MGMT* promoter and to estimate its impact on overall survival. Our cohort of samples included 33 diffuse glioma patients (21 male and 12 female). Positive methylation status was detected in 17 patients (51.5%) by methylation-specific polymerase chain reaction. Preliminary results obtained in our study suggest that hypermethylation of MGMT promoter was not found to correlate with overall survival. Significantly higher overall survival was associated with younger patients in comparison with the patients older than 50 years (19 months – 7 months median survival). It was also recorded that greater extent of tumor resection influences overall survival in patients with diffuse gliomas.
For more accurate evaluation, further evaluation of MGMT promoter methylation status on larger sample study is planned.

Poster presentation

## THE EPIGENETIC CHANGES AS POTENTIAL BIOMARKERS IN LIVER DISEASES INDUCED BY CHRONIC HEPATITIS C INFECTION WITH GENOTYPE 1B FROM SERBIA

Nikola Kokanov[1], Snežana Jovanović-Ćupić[1], Bojana Kožik[1], Ana Božović[1], Milena Krajnović[1]

[1]*University of Belgrade-Vinča Institute of Nuclear Sciences, Laboratory for Radiobiology and Molecular Genetics, Belgrade, Serbia*

Chronic hepatitis C virus infection is characterized by progressive fibrosis of variable degrees and long-term progression to cirrhosis and hepatocellular carcinoma. Prevention of chronic hepatitis C and its complications is based on antiviral therapy and early detection of reliable molecular markers in persons under the risk. This study was designed to describe the relationship between host and viral factors and their impact on response to therapy with pegylated interferon/ribavirin (PEG-IFN/RBV) in patients with chronic hepatitis C (HCV) infection with genotype 1b. Also, we investigated how IL28B single nucleotide polymorphism (SNP) rs12979860 and aberrant methylation of RASSF1A gene may have an impact on therapy response and stage of liver fibrosis individually and simultaneously. Samples were collected from a total 47 patients with chronic hepatitis C genotype 1b, before the start of PEG-IFN/RBV therapy. The SNP rs12979860 were determined by TaqMan assay. Methylation status of RASS1A gene were determined by chemical bisulfite modification of DNA and subsequent PCR, using primers specific for either methylated or unmethylated DNA. Methylated RASSF1A was found in 34% (16 of 47) samples. Among 26 patients with CC genotype of IL28B, RASSF1A methylation was detected in 50% (13 of 26) of samples, while among 21 patients with CT/TT genotype, RASSF1A methylation was detected in 14,3% (3/21) of samples. Observed differences was statistically significant (p=0.024). We also found significant association between response to therapy and rs12979860 genotypes (p= < 0.001). Thus, patients with CC genotype of IL28B rs12979860 were significantly more frequent in sustained virologic response (SVR) group than in non-response (NR) group. There was no correlation between rs12979860 genotypes, methylation status of RASSF1A and stage of liver fibrosis individually and simultaneously. Our results suggest that combined analysis of RASSF1A gene methylation status and IL28B rs1297860 polymorphism could help in prediction of therapy response.

Sunday, December 8th Session: ATOPY

Poster presentation

# HIGH PREVALENCE OF ANTI-THYROGLOBULIN IgE IN SUSPECTED ATOPIC AND NON-ATOPIC INDIVIDUALS

Tijana Trivić[1], Gordan Blagojević[1], Luka Dragačević[1], Rajna Minić[1]

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Thyroglobulin is a large glycoprotein which contains neutral sugars, sialic acid and iodine. In cow and pig, it also contains an α-gal epitope, which has been pinpointed as an epitope responsible for delayed immediate-type hypersensitivity to red meat.

The goal of this study was to test the reactivity of human serum IgE to unmodified commercial bovine and porcine thyroglobulin in ELISA. Two groups of subjects were tested; the people who were suspected to be atopic by general practitioners and professional athletes who declared themselves as not being atopic. Thyroglobulin specific IgE, IgG and IgG subclasses were tested in ELISA. IgE to commercial meat extract was also assessed in ELISA. Porcine thyroglobulin was separated according to charge on a DEAE matrix. The fractions were dot blotted and analyzed for IgE reactivity.

Among 229 suspected atopic subjects 27 (11.8%) had high IgE level and 41 (17.9%) had moderate level of thyroglobulin specific IgE. The situation was similar with professional athletes (n=38) 15,8% high and 23.7% moderate. The suspected atopic subjects who had high IgE level to thyroglobulin also had statistically higher meat extract specific IgE, but were not significantly different in any other antibody class/subclass tested. The IgG subclasses present, specific for thyroglobulin were IgG2 and IgG4.

Subjects who had detectable IgE levels were significantly younger (Hi: intermediary: low= 13: 13: 27 years; $p<0.0002$; Kruskal-Wallis). The IgE reactivity to porcine thyroglobulin is dependent on charge. The reactivity to α-Gal epitope cannot be assessed by using unmodified commercial bovine or porcine thyroglobulin in ELISA. The function of the detected anti-thyroglobulin IgE antibodies is unknown, but considering their preferential distribution in the younger age group they might come from the pool of natural IgE antibodies.

## Sunday, December 8th Session: ATOPY

Poster presentation

# 'SILENT' RESPIRATORY INFECTIONS IN ALGERIAN ADULT ASTHMATICS: PRELIMINARY DATA.

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Background. Asthma is a chronic inflammatory airway disease. Many factors have been reported to aggravate asthma symptoms including respiratory infections (RIs). These latter may cause asthma exacerbations and may lead to emergency room visits and hospital admission. Objectives. i) To investigate respiratory infections in asthmatic adults from Tizi-Ouzou city. ii) To determine antibiotic sensitivity of isolated germs. Methods. Asthmatic patients were recruited from the pulmonary department of the Mohamed Nedir university hospital after completing an asthma questionnaire. Sputum samples from 13 adult asthmatics without apparent symptoms for RIs and 5 healthy subjects were collected and sputum cytology and culture (bacterial and fungal infection) were performed. All identified bacteria were tested for antibiotic sensitivity to 16 antibiotics. Results. Sputum cytological analysis showed the presence of leucocytes cells including eosinophils in 7 samples (> 53%) from asthmatic patients. Epithelial cells were detected in 9 asthmatic samples (> 69%). Bacterial germs were found in 5 (> 38%) asthmatic samples including *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Citrobacter freundii*. However, *Candida albicans* was the only fungal germ found in 6 (> 46%) asthmatic samples. Interestingly, bacterial infection was found to be associated with high sputum total leukocyte values and high annual rate of asthma hospitalizations. No germs were found in non-asthmatic samples. Among tested antibiotics, only second generation cephalosporins (cefoxitin), aminoglycosides (gentamycin) and tetracyclines were effectives on all isolated bacteria. Conclusion. Our results show the presence of germs in the sputum of adult asthmatics without RIs symptoms. Moreover, RIs in adult asthmatics are associated with asthma hospitalization. Antibiotics may be a beneficial therapy to reduce asthma exacerbation and hospitalization. Sputum analysis and culture as a routine test in pulmonary department may help health professional to reduce asthma exacerbation.

Poster presentation

## TARGETING OF INFLAMMATION-CONNECTED TUMORS BY CARBORANE-CONJUGATED ROFECOXIB ANALOGS

Blagoje Murganić[1], Antonio Buzharevski[2], Svetlana Paskaš[1], Menyhárt-Botond Sárosi[2], Markus Laube[3], Peter Lönnecke[2], Wilma Neumann[2], Sanja Mijatović[1], Jens Pietzsch[3,4], Evamarie Hey-Hawkins[2], Danijela Maksimović-Ivanić[1]

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Connection between inflammation and tumorigenesis is very well described in the literature. Inducible cyclooxygenase-2 (COX-2), due to its important function in inflammation and tumor initiation/progression, is one of the potential targets in the treatment of inflammation-associated tumors. However, the application of both nonselective and selective COX-2 inhibitors revealed serious life threatening adverse effects. Recently, it was found that introduction of a carborane moiety into COX inhibitors significantly improved their potency against different cell lines. The aim of this study was to evaluate the efficacy of carborane-containing analogs of rofecoxib on a panel of melanoma and colon cancer cell lines. Novel carborane-containing analogs of rofecoxib significantly diminished the viability of tumor but not healthy mouse macrophages. Among them, the most efficient is a nitric oxide (NO) modified carborane-containing analog of rofecoxib. This compound strongly suppressed the proliferation of A375 cells without significant contribution of apoptotic cell death. The increase of both NO and reactive oxygen/nitrogen species was observed upon the treatment of A375 cells with this compound explaining its cytostatic potential. Moreover, the release of NO was not detected in culture medium and conditioned culture medium, indicating that this compound does not behave as exogenous donor. Since this carborane-containing analog of rofecoxib did not inhibit the COX-2 activity, its tumoricidal potential can be ascribed to a COX-independent action. In summary, the obtained results represent an excellent basis for further investigations of the antitumor potential of new classes of nonsteroidal anti-inflammatory drugs based on carborane-containing analogs of COX inhibitors in the treatment of inflammation-associated tumors.

Poster presentation

## COMBINED ACTION OF FULLEROL AND HYPERPOLARIZED LIGHT AGAINST iNOS+ AMELANOTIC HUMAN MELANOMA A375CELLS

Dijana Drača[1], Tamara Krajnović[1], Milica Markelić[2], Marija Slavković[3], Djuro Koruga[3], Sanja Mijatović[1], Danijela Maksimović-Ivanić[1]

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$C_{60}$ molecule - third allotropic form of elemental carbon in nature known as fullerene is the only molecule of a single element able to form a spherical cage. Inspired by the extraordinary optical properties of that molecule, new form of light defined by fullerene icosahedral symmetry is created using fullerene nanophotonic filter (Zepter). It is confirmed that hyperpolarized light (HPL) promotes micro-circulation and body's defense system. Additionally, the discovery of hydroxylated fullerenes, fullerol (3HFWC), with significantly improved solubility and, accordingly availability to biological systems, brought new possibility in the treatment of different pathologies. The aim of this study is to evaluate the influence of 3HFWC (Zepter) on highly invasive, anaplastic $iNOS^+$ human melanoma cells, A375, alone or in the combination with short, 15 min pulse of HPL. A375 cells were exposed to 3HFWC for 48h in wide spectrum of doses. Viability assessment, as well as microscopic evaluation, revealed significant decrease in the number of viable cells. Short term exposure to HPL remarkably potentiated effect of 3HFWC, while HPL alone didn't affect cell viability in the same setting. Determination of cell division rate in cultures exposed to $IC_{50}$ dose indicated strong blockage in proliferation, while massive presence of enlarged, floating cells suggested that the nano substance provoked specific type of cell death, anoikis. The expression of β galactosidase indicated that A375 cells obtained senescent phenotype. All these effects were accompanied with inhibited ROS/RNS production, while, interestingly, NO was significantly potentiated and probably responsible for the mentioned effects. Importantly, while the viability of primary peritoneal exudate cells was not disturbed by 3HFWC and 15 min long HPL exposure, combined treatment stimulated the proliferation of lymphocytes. Taken together, 3HFWC in combination with HPL might have beneficial effects on both tumor cells and immune system.

Poster presentation

## ANTITUMOR ACTIVITY OF GASTROPODAN HEMOCYANINS IN MURINE MODEL OF COLON CARCINOMA

E. Stoyanova[1], I. Manoylov[1], N. Mihaylova[1], K. Idakieva[2] and A. Tchorbanov[1,3]

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Introduction: The hemocyanins (Hcs) are oligomeric copper-containing glycoproteins that function as oxygen carriers in the hemolymph of several molluscs and arthropods. Molluscan Hcs have been studied intensively for many years as very promising class of anti-cancer therapeutic, generating strong humoral and cellular immune response. A possible mechanism for their antitumor effect is the presence of cross-reactive epitopes between the carbohydrate content of the hemocyanin molecule and tumor-associated carbohydrate antigens, which are characteristic for different types of cancer. Aim: The aim of the present work was to develop an experimental murine model of colon carcinoma and to investigate the anti-tumor activity of RtH and HpH. Materials and Methods: The Hcs were isolated from marine snail *Rapana thomasiana* (RtH) and the terrestrial snail *Helix pomatia* (HpH). Murine colon carcinoma cell line C-26 was used for animal administration and solid tumor establishment. Flow cytometry was performed for phenotyping of spleen and tumor suspensions and an apoptosis assay. The levels of cytokines and anti-C-26 antibodies were quantified by ELISA. Results: The Hcs exhibited strong *in vivo* anti-cancer and anti-proliferative effects in the developed murine model of colon carcinoma. We observed a significant increase of the spleens in non-treated C-26-bearing mice compared to Hcs treated. The immunization with RtH and HpH prolonged the survival of treated animals, improve humoral anti-cancer response and moderate the manifestation of C-26 carcinoma symptoms as tumor growth, splenomegaly and lung metastasis appearance. Conclusions: Hemocyanins are used so far for therapy of superficial bladder cancer and murine melanoma models. Our findings demonstrate a potential anti-cancer effect of hemocyanins on a murine model of colon carcinoma suggesting their use for immunotherapy of different types of cancer.

Poster presentation

## NOVEL PYRAZOLO[3,4-D]PYRIMIDINE DERIVATIVES SUPRESS P-GLYCOPROTEIN ACTIVITY AND REVERSE MULTIDRUG RESISTANCE IN CANCER CELLS

Jelena Dinić[1], Ana Podolski-Renić[1], Tijana Stanković[1], Maurizio Botta[2,3], Milica Pešić[1]

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P-glycoprotein (P-gp) is an ATP-binding cassette (ABC) transporter whose overexpression in cancer cells is one of the main causes of multidrug resistance (MDR). P-gp overexpression is responsible for reduced intracellular accumulation and efficacy of both targeted therapies and classic chemotherapeutics. Therefore, P-gp has an important role in "absorption, distribution, metabolism, and excretion" – ADME studies. It is also considered as the first cellular defense line and a part of so-called "cellular immunity'. Tyrosine kinase inhibitors (TKIs) have been reported to interact with ABC transporters, and in some cases increase the susceptibility of cancer cells to chemotherapy. We have investigated the anticancer potential of novel tyrosine kinase inhibitors pyrazolo[3,4-d] pyrimidines and their prodrugs against two pairs of sensitive and MDR cancer cell lines with P-gp overexpression: non-small cell lung carcinoma (NCI-H460 and NCI-H460/R) and colorectal carcinoma (DLD1 and DLD1-TxR). The tested compounds displayed significant cell growth inhibition and enhanced the efficacy of doxorubicin and paclitaxel in MDR cancer cells. Some of the TKIs directly interacted with P-gp and inhibited its ATPase activity. A kinetics study showed that the compounds increased the intracellular accumulation of the P-gp substrate rhodamine 123 in a time-dependent manner. Treatment with the compounds did not increase the mRNA expression level of P-gp in resistant cancer cells. The investigated pyrazolo[3,4-d] pyrimidines showed significant potential for reversing P-gp-mediated MDR even in prolonged treatment, making them good candidates for further development regarding treatment of resistant cancers.

Poster presentation

## EFFECT OF NON-CYTOTOXIC DOSES OF CADMIUM ON THE B16 MELANOMA CELL LINE

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Cadmium (Cd) is one of the most cytotoxic agents and environmental contaminants, which can cause serial health problems in various organs such as liver, kidney, testis, bone, nervous tissue and immune system. Furthermore, Cd is classified as a human and animal carcinogen agent. Despite the fact that Cd accumulates in the skin, there is a lack of evidence about its involvement in skin cancer biology. Skin cancer is one of the most common cancers worldwide, with melanoma as the most lethal type. Based on that, we set up the study to examine the effect of cadmium on the B16 melanoma cell line, measuring viability by MTT and crystal violet (CV) tests, proliferation rate (CFSE), ROS production (DHR), migration (scratch test) and adhesion on matrigel. The results showed that non-cytotoxic doses of cadmium (ranging from 0,3-2,5μM) increased viability, proliferation, and migration of B16 cells, while at the same time slightly promoted ROS production. Otherwise, pronounced oxidative stress induced by higher doses (ranging from 5-20μM) led to cell death. Summary, these results showed that low doses of cadmium could upregulate B16 melanoma cell line growth and migration probably through moderate ROS/RNS generation and thus their ability to modulate relevant signaling pathways involved in cancer progression. Considering that, underlying mechanisms of Cd impact on melanoma cell lines proliferation, migration and invasiveness needs to be clarified *in vitro*, as well as its relevance for *in vivo* system.

The work is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant #173013 and #173039

Poster presentation

## SINERGISTIC EFFECTS OF FULLERENE WATER COMPLEX AND HYPERPOLARIZED LIGHT AGAINST B16-F10 MELANOMA CELLS

Milica Markelić[1], Dijana Drača[2], Tamara Krajnović[2], Marija Slavković[3], Djuro Koruga[3], Sanja Mijatović[2], Danijela Maksimović-Ivanić[2]

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Thanks to their unique cage-like structure and electron-deficiency, carbon nanomaterials fullerenes are in the focus of research in many fields, including medicine. An important task for the application of fullerenes in therapy of different pathologies, including cancer, is to achieve their water solubility. By addition of hydroxyl groups, a Hyper-harmonized Fullerene Water Complex (3HFWC, Zepter) with significantly improved solubility and bioavailability was patented. The fullerene-based nanotechnology was recently applied in light therapy, by the application of fullerene nanophotonic filter in BIOPTRON® device (Zepter), thus changing the properties of light and converting it to hyperpolarized light (HPL). The beneficial effects of HPL on micro-circulation, wound healing and immune system is already confirmed. The aim of this study was to investigate the antitumor activity of 3HFWC on melanoma cells *in vitro*, alone or in the combination with HPL. For this purpose, B16-F10 mouse melanoma cells were exposed to 3HFWC for 48h (IC50 dose - 50 μg/ml). In order to evaluate HPL effects, single, short term irradiation of cells with HPL was applied. Our results on determination of cell division rate as well as microscopic analysis, clearly demonstrated inhibition of proliferation, without significant increase of cell death. Although HPL alone did not affect significantly proliferation rate of melanoma cells, our study showed that it potentiated 3HFWC effects. Additionally, senescent phenotype was stimulated during these treatments, as confirmed by β galactosidase expression, the appearance of giant mitochondrial and accumulation of lipid bodies. Additional ultrastructural study demonstrated internalization of 3HFWC aggregates by endocytosis. In conclusion, our results clearly demonstrate antitumor effects of 3HFWC on B16-F10 melanoma cells, which is stimulated by HPL. Thus, their combination presents potentially promising strategy in cancer therapy.

Poster presentation

## MAP KINASE-DEPENDENT AUTOPHAGY IS INVOLVED IN PHORBOL MYRISTATE ACETATE DIFFERENTIATION OF LEUKEMIA CELLS INTO MACROPHAGE-LIKE CELLS

Miloš Mandić[1], Maja Misirkić-Marjanović[2], Ljubica Vučićević [2], Maja Jovanović[3], Mihajlo Bošnjak[4], Vladimir Perović[1], Ljubica Harhaji-Trajković[2] and Vladimir Trajković[1]

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Aim: We investigated the mechanism and the role of autophagy in phorbol myristate acetate (PMA)-induced myeloid differentiation of human acute myeloid leukemia HL-60 cells. Methods: The mRNA levels of myeloid differentiation markers colony stimulating factor 1 receptor (CSF1R), early growth response protein 1 (EGR1), and interleukin 8 (IL-8), were assessed by real-time RT-PCR. Cell cycle arrest and the expression of surface myeloid marker CD11b and CD45 were analyzed by flow cytometry. Autophagy was monitored by acridine orange staining, RT-PCR analysis of autophagy-related (ATG) gene expression, immunoblot analysis of LC3-II/p62, Beclin-1/Bcl-2 interaction, nuclear translocation of transcription factor EB (TFEB) and Foxo3. The activation of MAP kinases extracellular signal-regulated kinase (ERK) and c-Jun-N terminal kinase (JNK) was assessed by immunoblotting. Pharmacological inhibition and RNA interference (RNAi) were used to determine the role of MAP kinases in autophagy and HL60 cell differentiation, while the role of autophagy in HL60 differentiation was analyzed using RNAi-mediated knockdown of ATG5 and p62. Results: PMA-induced differentiation of HL-60 cells into macrophage-like cells was confirmed by cell-cycle arrest accompanied by elevated expression of p21, CD11b, CD45, CSF1R, EGR1, and IL-8. The induction of autophagy was demonstrated by accumulation of LC3-II, the increase in autophagic flux, the increase in expression of ATG genes, nuclear translocation of TFEB and Foxo3 and dissociation of Beclin1from Bcl-2. The suppression of autophagy by RNAi–mediated knockdown of ATG5 or p62 counteracted myeloid differentiation of HL60 cells. Both ERK and JNK were activated by PMA, and their pharmacological and genetic inhibition decreased PMA-induced autophagy and differentiation of HL60 cells. Conclusion: Our study revealed the involvement of JNK and ERK in autophagy-dependent differentiation of HL60 cells into macrophage-like cells, indicating MAP kinase-mediated autophagy as a possible target for the treatment of acute myeloid leukemia.

Sunday, December 8th Session: CANCER

Poster presentation

## REMODELLING OF EXTRACELLULAR MATRIX IN THE UNINVOLVED HUMAN COLON MUCOSA 10 CM AND 20 CM AWAY FROM THE MALIGNANT TUMOR

Sanja Despotović[1], Novica Milićević[1], Živana Milićević[1], Aleksandar Krmpot[2], Mihailo Rabasović[2]. Aleksandra Pavlović[3], Vladimir Živanović[3]

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Changes in morphology and organization of extracellular matrix (ECM) in cancer, itself, are the subject of numerous studies, while it is far less known about the changes in the uninvolved mucosa away from cancer. The aim of our study was to analyse changes in morphology and organization of ECM components in the uninvolved colonic mucosa 10 cm and 20 cm away from the cancer, in comparison with healthy subjects.Tissue samples 10 cm and 20 cm away from the colon cancer, were obtained during colonoscopy from 25 patients and 30 healthy controls at the University Hospital Center "Dr Dragiša Mišovic-Dedinje". For collagen fiber visualization and analysis nonlinear laser scanning microscopy with SHG detection was used. CT-FIRE and CURVE-ALIGN were used to assessed individual collagen fibers and calculate length, straightness, width and alignment. The anisotropy parameter β was calculated on the same microscope with addition of polarizer. For analysis of reticular fibers Gomori's silver impregnation technique was used. For visualization of periostin and hyaluronic acid immunohistochemical staining with anti-periostin and anti-HABP antibody was used. In the Fiji and Icy softwares we calculated representation of extracellular matrix components and diameter of spaces between them. At the distance 10 cm and 20 cm away from the cancer, representation of ECM components was significantly decreased, compared with healthy mucosa. The diameter of spaces between ECM components were significantly increased. Also, we detected increase in length, width, straightness and alignment of collagen fibers at the distance 10 cm and 20 cm away from the cancer compared with healthy colon lamina propria. Using different, complementary approaches we detected changes in morphology and organization of ECM components at the distance 10 cm and 20 cm away from colon cancer, compared with healthy subjects.

Poster presentation

## EFFECTS OF PACLITAXEL ON TGF-β-INDUCED UROKINASE-TYPE PLASMINOGEN ACTIVATOR EXPRESSION AND MIGRATION OF RAW 264.7 MACROPHAGES

Sonja Mojsilović[1], Slavko Mojsilović[2], Milica Tošić[3], Marija Živanović[3], Sunčica Bjelica[3], Tatjana Srdić-Rajić[4], Juan F. Santibañez[3,5]

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Paclitaxel (PTx), a natural diterpene alkaloid, is a widely used anticancer drug with immune-activating properties. It irreversibly enhances polymerization of microtubules (MT), disrupting tubulin cytoskeleton dynamics, leading to cell migration inhibition, cell cycle arrest, and cell death. It was shown that PTx reduce macrophage (Mφ) tumor infiltration and induce Mφ M1 polarization. On the other hand, transforming growth factor-β (TGF-β) is highly abundant in tumor in tumor microenvironment plays a profound role in regulating Mφ function and tumor growth. It induces alternative Mφ activation that favors immunosuppression and tumor progression. TGF-β also increases macrophage recruitment to tumor stroma by mechanisms that include enhancement of cell motility and extracellular matrix degradation. MT integrity is crucial for TGF-β intracellular signaling, which suggests that PTx may be useful for targeting MT cytoskeleton and regulating signaling and functions of TGF-β in Mφ.

In this study we used mouse macrophage RAW 264.7 cells treated with combinations of PTx and TGF-β. Proliferation was analyzed by MTT assay and cell cycle analysis. Immunofluorescence was performed to determine tubulin cytoskeleton and Smad3 nuclear localization. Western blot and transcriptional luciferase reporters were used to measure signal transduction activation. Migration was determined by wound-healing assay. uPA activity was determined by zymography assay. We found that PTx decreased RAW 264.7 cell proliferation by inducing G2/M cell cycle arrest and profoundly modified the tubulin cytoskeleton. Also, PTx inhibited TGF-β-induced Smad3 activation. Furthermore, PTx decreased cell migration and uPA expression stimulated by TGF-β. Remarkably, p38 MAPK mediated PTx inhibition of uPA activity induced by TGF-β, but it was not implicated in cell migration inhibition. In conclusion, PTx inhibits TGF-β induction of mouse Mφ migration and uPA expression, suggesting that PTx, as TGF-β targeting therapy, may enhance Mφ anticancer action within tumors. *Mojsilovic S, Tosic M, Mojsilovic S, Zivanovic M, Bjelica S, Srdic-Rajic T, Santibanez JF. Paclitaxel inhibits transforming growth factor-β-increased urokinase-type plasminogen activator expression trough p38 MAPK and RAW 264.7 macrophage migration. J BUON (in press)

Sunday, December 8th Session: CANCER

Poster presentation

# POLYMORPHISMS AND EXPRESSION OF OMEGA CLASS GLUTATHIONE TRANSFERASES IN CLEAR CELL RENAL CELL CARCINOMA

Tanja Radic[1], Vesna Coric[1], Tatjana Djukic[1], Smiljana Mihailovic[2], Marija Pljesa-Ercegovac[1], Marija Matic[1], Dejan Dragicevic[3], Tatjana Simic[1,4], Ana Savic-Radojevic[1]

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Omega class glutathione transferases, GSTO1-1 and GSTO2-2, exhibit a distinctive range of different activities involved in regulation of inflammation, apoptosis and cellular redox homeostasis. Significant changes in those cellular processes occur in clear cell renal cell carcinoma (ccRCC). We aimed to determine the potential effect of *GSTO1**C419A (rs4925), *GSTO2**A424G (rs156697) and *GSTO2**A183G (rs2297235) polymorphisms on postoperative prognosis in ccRCC patients. Furthermore, in non-tumor and tumor ccRCC tissue GSTO1-1 and GSTO2-2 expression, as well as, phosphorylation status of PI3K/Akt/mTOR and Raf/MEK/ERK signaling pathways was assessed. Considering the role of GSTO1 in IL1-β posttranslational processing, pro-IL-1β and IL-1β levels in ccRCC tissue samples were determined. Additionally, we investigated possible association of GSTO1 with signaling molecules known to be regulated by glutathionylation. *GSTO1* (rs4925) and *GSTO2* (rs156697, rs2297235) genotyping was performed in 239 ccRCC patients, while ccRCC tumor and non-tumor specimens were used for immunoprecipitation and immunoblot using anti-GSTO1, Akt, phospho-Akt (pT308), β-actin antibodies and Akt/MAPK signaling pathway antibody cocktail. Pro-IL-1β and IL-1β levels in tumor ccRCC tissue samples were determined by ELISA. We showed that *GSTO1**CC (rs4925) genotype, exhibiting higher deglutathionylase activity, predicts shorter survival among male patients (p=0.049). Additionally, male carriers of *GSTO1**CC (rs4925) genotype had significantly increased mortality risk compared to the carriers of *GSTO1**A allele (p=0.037). Higher protein expression of GSTO1-1 and GSTO2-2 has been shown in tumor ccRCC compared to their respective non-tumor specimens (p=0.002, p=0.007, respectively). Expression of phosphorylated proteins of Akt/MAPK signaling pathway (p90RSK1 (pS380), Akt (pS473) and ERK1/2 (pY204/187)) was increased in ccRCC tumor compared to corresponding non-tumor specimens. Furthermore, immunoprecipitation has shown an association of GSTO1 with Akt, phospho-Akt, p90RSK1 (pS380) and RPS6 (pS235/236). Weak positive correlation was found between GSTO1 protein expression and IL-1β/ pro-IL-1β ratio (r=0.260, p=0.350). In conclusion *GSTO1**CC genotype might be determinant of postoperative prognosis among male ccRCC patients.

Poster presentation

## MODULATION OF FUNCTIONAL CHARACTERISTICS OF MURINE PERITONEAL MACROPHAGES BY DEHYDROGENATE POLYMER FROM CONIFERYL ALCOHOL AND ALGINATE

Ana Kovačević[1], Ivana Lukić[1], Emilija Marinković[1], Radmila Miljković[1], Aleksandra Inić-Kanada[2], Dragica Spasojević[3], Ksenija Radotić[3], Marijana Stojanović[1]

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The dehydrogenate polymer from coniferyl alcohol (DHP; a lignin model compound) in alginate hydrogel (ALG) has been shown to exert a strong antibacterial activity. To broadens a spectrum of potential DHP/ALG application, we aimed this study to evaluate the immunomodulatory activity of DHP/ALG. DHP and ALG were tested separately and in mixture (1:2 w/w) for their impact on *in vitro* production of cytokines (IL-6, IL-12, and IL-10) and reactive oxygen (ROS) and nitrogen (RNS) species by resident (RMs) and thioglycolate-elicited (TGMs) peritoneal macrophages of BALB/c mice. RMs and TGMs were stimulated (48h) with ALG and DHP in concentrations previously shown to be non-cytotoxic (up to 50 and 25 μg/ml, respectively). DHP/ALG promotes simultaneous production of inflammatory (IL-6, IL-12) and regulatory cytokines by RMs in a positive dose-dependent manner. Production of inflammatory cytokines was stimulated by ALG, while an increase in IL-10 production positively correlated to the concentration of DHP. ALG also stimulated the production of IL-12 by TGMs, which was mirrored in the outcome of ALG/DHP stimulation. The significant increase in the activity of myeloperoxidase (MPO) due to DHP and/or ALG stimulation was recorded in TGMs, while a slight increase in MPO activity in RMs was recorded only upon stimulation with the higher amount of ALG. ALG in a positive dose-dependent manner stimulated the production of ROS and RNS by both RMS and TGMs. In all cases, except ROS production by RMs, the impact of ALG stimulation was mirrored in the outcome of ALG/DHP stimulation. Our results suggest that DHP/ALG exerts an immunomodulatory activity that could complement already reported antimicrobial activity and warrants further investigation on the use of DHP/ALG in the treatment of infectious diseases. (Supported by Ministry of Education, Science and Technological Development Republic of Serbia, grants 172049 and 173017)

Poster presentation

# ARONIA BERRIES FRUIT WATER EXTRACT STIMULATES CELLS OF THE IMMUNE SYSTEM *IN VITRO* AND *IN VIVO*

Dragica Gajić[1], Tamara Saksida[1], Ivan Koprivica[1], Milica Vujicic[1], Sanja Despotovic[2], Katarina Savikin[3], Teodora Jankovic[3], Ivana Stojanovic[1]

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Many plant extracts are well known for their anti-oxidant, anti-bacterial and anti-inflammatory activities including Aronia berry-derived juices and powders. In comparison to other black berries, Aronia berries have a greater content of phenolic constituents such as procyanidins, anthocyanins and phenolic acids with antioxidative and anti-inflammatory properties. However, the effects of aronia berries extract on the immune response parameters have been only sporadically assessed. When administered orally to healthy C57BL/6 mice (50 mg/kg body weight), aronia extract exerted immunomodulatory effects as evidenced by decreased proportion of $F4/80^+$ macrophages, $CD11c^+$ dendritic cells, $CD4^+$ T helper cells, $CD8^+$ T cytotoxic lymphocytes and $CD4^+CD25^-$ activated lymphocytes within the gut-associated lymphoid tissue. Surprisingly, oral consumption of chokeberry extract in doses of either 200 mg/kg bw or 50 mg/kg bw in mice with multiple low dose streptozotocin-induced type 1 diabetes resulted in the increase of blood glucose levels. Further, our study shows that this detrimental effect on type 1 diabetes pathogenesis may be a consequence of the pro-inflammatory nature of the extract. This is based on the evident stimulation of macrophages and dendritic cells by the extract through up-regulation of pro-inflammatory mediators such as nitric oxide, IL-12, IL-6 and TNF *in vitro*. Also, this extract augmented differentiation of IFN-γ-producing T helper 1 cells *in vitro*. Collectively, the obtained results imply that our particular aronia berries fruit extract displays pro-inflammatory characteristics and that care should be taken when these berries are to be included in the human diet.

Sunday, December 8th Session: CELLS

Poster presentation

# EVALUATION OF THE IMMUNOSAFETY OF CUCURBIT[7]URIL *IN VITRO* ON PERIPHERAL BLOOD MONONUCLEAR CELLS

Ekaterina Pashkina[1,2], Alina Aktanova[1], Elena Blinova[1], Alexandr Ermakov[2], Vladimir Kozlov[1,2]

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Cucurbiturils (CB[n]) are nanoscale macrocyclic compounds capable of encapsulating a molecule or a fragment of the molecule drug compounds by forming host-guest complexes. Integration of drugs with cucurbituril used for the following purposes: to control clearance, protection of the drug from the biodegradation of targeted delivery to specific organs, tissues or cells, reduce toxicity, improve the solubility, etc. However, one of the major problems encountered in the application of new drug delivery systems is the study of their biological properties. Today it is known that CB[n], unlike many other often toxic nanoparticles, have extremely low toxicity in high doses. However, many aspects of the biological actions of these nanoscale cavitands, including immunotropic properties remain unclear. The aim of this study was to investigate immunotoxicity and immunomodulation properties of CB[7]. It was found that CB[7] did not decrease of viability of PBMCs in all used concentrations from 0.1 to 1 mM. In addition, 0,5 mM concentration of CB[7] increase the number of T-helpers,but decrease in the expression of activation markers (CD69, HLA-DR) on T cells. Overall, results indicate immunomodulatory effect of high concentrations of CB[7]. The reported study was funded by Russian Science Foundation according to the research project ?19-15-00192.

Poster presentation

## 17β-ESTRADIOL AND GENISTEIN AFFECT MACROPHAGE INFLAMMATORY CYTOKINE PRODUCTION DURING AGING IN SEX-SPECIFIC MANNER

Ivana Ćuruvija[1], Stanislava Stanojević[1], Veljko Blagojević[1], Raisa Petrović[1], Ivana Prijić[1] and Vesna Vujić[2]

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Aging differently affects the expression of estrogen receptors alpha and beta (ERα and ERβ) and Toll-like receptors (TLR4) on peritoneal cavity cells of male and female rats. We explored the involvement of ERα and ERβ in the *in vitro* treatment of LPS-stimulated peritoneal macrophages with 17β-estradiol (which stimulates both receptors) or genistein (which is predominantly an ERβ agonist) on inflammatory cytokine secretion from young (3 months old) and middle-aged (16 months old) female and male AO rats. Aging diminished the proportion of TLR4+ cells and secretion of IL-1β and IL-6 in macrophages from female rats while the effect on male rat macrophages was opposite. 17β-estradiol increased IL-1β secretion by middle-aged females' macrophages *via* ERα, and suppressed it in cells from young females *via* ERβ. Genistein-induced decrease of IL-1β in macrophages from all experimental groups was probably mediated by ERβ. 17β-estradiol augmented IL-6 secretion by cells from all experimental groups *via* ERα while genistein diminished it in all females' and in middle-aged male rats' macrophages by activating ERβ. However, genistein increased IL-6 secretion from macrophages of young male rats *via* ERα. Although 17β-estradiol and genistein stimulated secretion of macrophage inflammatory cytokines *via* ERα and suppressed it probably *via* ERβ, their modulatory actions were determined by aging-induced changes in macrophage ERs expression and possible ERα / ERβ interactions (Supported by Ministry of Education, Science and Technological development, Republic of Serbia, Grant No 175050).

Sunday, December 8th Session: CELLS

Poster presentation

## KOREAN RED GINSENG EXHIBITS ANTI-INFLAMMATORY EFFECTS IN MICROGLIAL CELLS

Ji-Hee Kim[2], Ji Eun Shin[1], Kyungmin Lee[1], Iskander Madhi[1], Ruth Sangare[1], YoungHee Kim[1,2]

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Korean red ginseng (KRG) has long been used as a traditional herbal medicine with various pharmacological effects. In this study, KRG was shown to reduce lipoteichoic acid (LTA)-induced nitric oxide (NO) secretion and inducible nitric oxide synthase (iNOS) expression in BV-2 microglial cells without cytotoxicity. And it also suppressed nuclear translocation of NF-κB p65 and degradation of IκB-α. Furthermore, heme oxygenase-1 (HO-1) protein was dose-dependently increased by KRG and this induced HO-1 suppressed iNOS expression. In addition, KRG-mediated HO-1 expression was suppressed by the inhibitors of phosphoinositide-3-kinase (PI-3K) and mitogen-activated protein kinases (MAPKs), and KRG induced the phosphorylation of these kinases. Thus, these results suggest that KRG might be very useful treatment for Gram-positive bacteria-induced neuroinflammation and also have therapeutic potential for various neuro-inflammatory diseases.

Sunday, December 8th Session: CELLS

Poster presentation

## EXOGENOUS ALPHAKETOGLUTARATE IMPAIR DIFFERENTIATION AND MATURATION OF HUMAN DENDRITIC CELLS

Marijana Milanović[1], Marina Bekić[2], Dragana Vučević[1], Miodrag Čolić[2], Sergej Tomić[2]

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Alphaketoglutarate (aKG) is a key intermediate in cell metabolism. Exogenous aKG was shown to extend the lifespan by regulating the cellular response to callory restriction (CR). However, it is not known how the exogenous aKG affects immune responses, particularly those mediated by key immunoregulatory cells, dendritic cells (DC). Using a model of human monocyte-derived DC, we found that exogenous aKG (10mM or 50mM) does not induce autophagy in DC (according to LC3II expression), but impairs the differentiation of DC from monocytes (acording to CD1a and CD14 co-expression). Additionally, aKG inhibited LPS/IFN-γ-induced upregulation of NLRP-3, IL-1β, HLA-DR, CD83, CD86, CD40, CCR7, CD209, IL-33, IL-10 and IL-12p70 expression in DC, but potentiated the capacity of these cells to express TGF-β, CD73, and IL-23 in a dose-dependent manner. Although aKG-treated DC, displayed a lower capacity to stimulate proliferation of alloreactive T cells compared to control mature DC, the normalized number of $CD4^+ROR\gamma t^+IL17^+$ (Th17) and $CD4^+GATA3^+IL\text{-}4^+$ (Th2) cells was higher, and the number of $CD4^+$T-$bet^+IFN\text{-}\gamma^+$ (Th1) and $CD8^+IFN\text{-}\gamma^+GranzA^+Perf^+$ (CTL) was lower in co-cultures with aKG-treated DC, compared to corresponding control DC. Morover, aKG increased significantly the capacity of DC to induce $CD4^+CD25^{hi}CD127^-FoxP3^+$ Tregs in a dose dependent manner. These results suggested that, while exogenous aKG could have beneficial effects on lifespan, the quality of life might be compromized due to its immunomodulatory effects related to reduction of Th1 mediated immune responses.

Sunday, December 8th Session: CELLS

Poster presentation

# FUNCTIONALIZATION-DEPENDENT MODULATION OF HUMAN DENDRITIC CELLS BY WOOD-BASED CELLULOSE NANOCRYSTALS

Miloš Vasiljević[1], Marina Bekić[2], Bojan Joksimović[1], Dušan Mihajlović[3], Marijana Milanović[3], Ivana Majstorović[3], Dragana Vučević[1,3], Miodrag Čolić[1,2,3], Sergej Tomić[2,3]

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Cellulose nanocrystals (CNC) are attractive nanomaterials with large surface area suitable for development of drug delivery and diagnostics systems. However, the biocompatibility and immunomodulatory properties of CNC have not been studied so far. Here we used wood-based native (n)CNC, TEMPO-oxidized (o)CNC and phosphonated (p)CNC, to assess their toxicity and immunomodulatory potential on human monocyte derived dendritic cells (MoDC) *in vitro*. We found that non-toxic concentrations CNC impair the differentiation of MoDC, according to CD14/CD1a co-expression analysis. oCNC displayed the strongest inhibitory effect on MoDC differentiation, followed by nCNC and pCNC, respectively. These results correlated with the weakest maturation capacity of oCNC-treated MoDC upon stimulation with LPS/IFN-γ. Additionally, nCNC- and oCNC-treated MoDC expressed higher levels of PD1-L, TGF-β and ILT-4 compared to control MoDC, whereas pCNC-treated MoDC showed no such phenotypic properties. The capacity of MoDC to produce higher levels of IL-12p70, IL-1β, IL-23, and low levels of IL-10, were impaired by nCNC and oCNC, but not by pCNC. In line with this, nCNC- and oCNC-treated MoDC displayed an increased capacity to induce alloreactive Th2 cells, and TGF-β-producing $CD4+CD25^{hi}FoxP3+$ Treg cells, and a decreased capacity to induce IFN-γ-producing Th1 cells in co-culture. Cumulatively, these results suggest that CNC may induce tolerogenic properties in MoDC, whereas phosphonation of CNC prevents such an effect, thus restoring the immunogenic potential of MoDC.

Poster presentation

## THE EFFECT OF THE BRAFV600E MUTATION ON THE LEVEL OF EXPRESSION OF BANCR IN PAPILLARY THYROID CARCINOMA

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Papillary thyroid carcinoma (PTC) is the most common malignant tumor of the endocrine system. However, little is known about the genetic mechanisms underlying the development of PTC. BRAFV600E is the most common somatic mutation found in PTC that confers constitutive activation of the MAPK/ERK pathway. BRAF-activated long non-coding RNA (BANCR) is a novel and potential regulator of cancer cell proliferation and survival. But, findings concerning BANCR expression and its function in thyroid carcinomas are controversial. The aim of the present study was to explore the possible influence of the BRAFV600E mutation on BANCR level of expression in PTC. The level of expression of BANCR in PTC and matched nonmalignant thyroid epithelial tissues from 54 patients was determined using quantitative RT-PCR. Mutant allele-specific PCR was used for the detection of the BRAFV600E mutation presence. In the total sample, there is noticeable decrease of BANCR expression in PTC vs. adjacent nonmalignant thyroid tissue, but the down-regulation is not statistically significant. Conversely, the relative level of expression of BANCR in PTC depends on the presence of the BRAFV600E mutation, so the presence of the BRAFV600E mutation associates with lower relative BANCR expression in PTC ($p=0.011$). Furthermore, the fold change of BANCR expression in PTC vs. matched nonmalignant thyroid epithelial tissues depends on the presence of the BRAFV600E mutation ($p=0.003$). In the BRAFV600E positive PTC, the level of expression of BANCR decreases significantly compared to its levels in adjacent nonmalignant thyroid tissue, while in the BRAFV600E negative PTC, the relative level of expression of BANCR is unchanged or higher compared to the value for paired nonmalignant thyroid tissue. This implies that further research on the role of BANCR in the pathogenesis of PTC should include the BRAFV600E mutation status.

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