Plovdiv University "Paisii Hilendarski"

Annotation
of the materials under art. 65 (1) of the Regulations for development of academic staff of PU "Paisii Hilendarski", including self-evaluation of overall contributions and input

of **ch. assist. prof. Tsvetelina Georgieva Batsalova, PhD**

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Participant in the competition for the academic position "Associate Professor" in the field of higher education **4. Natural Sciences, Mathematics and Informatics**, professional field **4.3. Biological sciences**, scientific specialty **Cell Biology**, announced in the State Gazette, **issue 57/26.06.2020**

I. Compliance with the conditions for holding the academic position of "Associate Professor" under Art. 65. (1) of the regulations for development of the academic staff of PU “P. Hilendarski "

Since 2011 I have been teaching/lecturing at Plovdiv University “P. Hilendarski ”and I am part of the team of the Department of Developmental Biology. In 2012 I obtained my PhD in Cellular Biology. I have been holding the academic position "Chief Assistant Professor" since 2013.

To participate in the competition for the academic position "Associate Professor" in the scientific specialty Cell Biology, I hereby present a total of 21 scientific works that have not been used in the procedures for obtaining the educational and scientific degree "Doctor" and for the academic position of "Assistant Professor". They are classified into the following groups (in accordance to the list of scientific papers):

- ✓ 14 publications in journals with impact factor;
- ✓ 3 publications in scientific journals without impact factor;
- ✓ 2 publications in proceedings of conferences and congresses;
- ✓ 2 textbooks.

All scientific papers submitted for review are co-authored, 19 of them are written in English, 2 are written in Bulgarian. I am a leading author in 10 of the presented scientific articles.

SCIENTIFIC PUBLICATIONS IN JOURNALS WITH IMPACT FACTOR

1. **Batsalova T.**, Dzhambazov B., Klaczkowska D., Holmdahl R. 2010. Mice producing less reactive oxygen species are relatively resistant to collagen glycopeptide vaccination against arthritis. J. Immunol., 185(5): 2701-2709. (2010 impact factor – 5.745)

Abstract: The bottleneck for the induction of collagen-induced arthritis in mice is the recognition of immunodominant type II collagen (CII) peptide (CII259-273) bound to the MHC class II molecule Aq. We have shown previously that the posttranslationally glycosylated lysine at position 264 in this epitope is of great importance for T cell recognition and tolerance induction to CII as well as for arthritis development. The Ncf1 gene, controlling oxidative burst, has been shown to play an important role for immune tolerance to CII. To investigate the effect of oxidation on the efficiency of immune-specific vaccination with MHC class II/glycosylated–CII peptide complexes, we used Ncf1 mutated mice. We demonstrate that normal reactive oxygen species (ROS) levels contribute to the establishment of tolerance and arthritis protection, because only mice with a functional oxidative burst were completely protected from arthritis after administration of the glycosylated CII259–273 peptide in complex with MHC class II. Transfer of T cells from vaccinated mice with functional Ncf1 protein resulted in strong suppression of clinical signs of arthritis in B10.Q mice, whereas the Ncf1 mutated mice as recipients had a weaker suppressive effect, suggesting that ROS modified the secondary rather than the primary immune response. A milder but still significant effect was also observed in ROS deficient mice. During the primary vaccination response, regulatory T cells, upregulation of negative costimulatory molecules, and increased production of anti-inflammatory

versus proinflammatory cytokines in both Ncf1 mutated and wild type B10.Q mice was observed, which could explain the vaccination effect independent of ROS.

2. **Batsalova T.**, Lindh I., Backlund J., Dzhambazov B., Holmdahl R. 2012. Comparative analysis of collagen type II-specific immune responses during development of collagen-induced arthritis in two B10 mouse strains. Arthritis Res. Ther. 14(6):R237. (2012 impact factor – 4.3)

Abstract: Immune responses against collagen type II (CII) are crucial for the development of collagen-induced arthritis (CIA). The aim of the present study was to evaluate and compare the CII-directed T cell and antibody specificity at different time points in the course of CIA using two mouse strains on the B10 genetic background - B10.Q, expressing A^q MHC class II molecules, and B10.DR4.Ncf1$^{*/*}$, expressing human rheumatoid arthritis-associated MHC II DR4 molecules (DRA*0101/DRB*0401). B10.Q and B10.DR4.Ncf1$^{*/*}$ mice were immunized with CII emulsified in adjuvant and development of CIA was assessed. T cells from draining lymph nodes were restimulated in vitro with CII peptides and interferongamma (IFN-γ) levels in culture supernatants were evaluated by ELISA. CII-specific antibody levels in serum samples were measured by ELISA. At four different CIA time points we analyzed T cell specificity to the immunodominant CII epitope 259-273 (CII259-273) and several posttranslationally modified forms of CII259-273 as well as antibody responses to three B cell immunodominant epitopes on CII (C1, U1, J1). Our data show that CII-specific T and B cell responses increase dramatically after disease onset in both strains and are sustained during the disease course. Concerning anti-CII antibody fine specificity, during all investigated stages of CIA the B10.Q mice responded predominantly to the C1 epitope, whereas the B10.DR4.Ncf1$^{*/*}$ mice also recognized the U1 epitope. In the established disease phase, T cell reactivity toward the galactosylated CII259-273 peptide was similar between the DR4- and the A^q-expressing strains whereas the response to the non-modified CII peptide was dramatically enhanced in the DR4 mice compared with the B10.Q. In addition, we show that the difference in the transgenic DR4-restricted T cell specificity to CII259-273 is not dependent on the degree of glycosylation of the collagen used for immunization. The present study provides important evaluation of CII-specific immune responses at different phases during CIA development as well as a comparative analysis between two CIA mouse models. We indicate significant differences in CII T cell and antibody specificities between the two strains and highlight a need for improved humanized B10.DR4 mouse model for rheumatoid arthritis.

3. Andersson I.E., Andersson C.D., **Batsalova T.**, Dzhambazov B., Holmdahl R., Kihlberg J., Linusson A. 2011. Design of glycopeptides used to investigate class II MHC binding and T-cell responses associated with autoimmune arthritis. PLoS ONE, 6(3):e17881. (2011 impact factor – 4.092)

Abstract: The glycopeptide fragment CII259–273 from type II collagen (CII) binds to the murine A^q and human DR4 class II Major Histocompatibility Complex (MHC II) proteins, which are associated with development of murine collagen-induced arthritis (CIA) and rheumatoid arthritis (RA), respectively. It has been shown that CII259–273 can be used in therapeutic vaccination of CIA. This glycopeptide also elicits responses from T-cells obtained from RA patients, which indicates that it

has an important role in RA as well. We now present a methodology for studies of (glyco)peptide-receptor interactions based on a combination of structure-based virtual screening, ligand-based statistical molecular design and biological evaluations. This methodology included the design of a CII259–273 glycopeptide library in which two anchor positions crucial for binding in pockets of A^q and DR4 were varied. Synthesis and biological evaluation of the designed glycopeptides provided novel structure-activity relationship (SAR) understanding of binding to A^q and DR4. Glycopeptides that retained high affinities for these MHC II proteins and induced strong responses in panels of T-cell hybridomas were also identified. An analysis of all the responses revealed groups of glycopeptides with different response patterns that are of high interest for vaccination studies in CIA. Moreover, the SAR understanding obtained in this study provides a platform for the design of secondgeneration glycopeptides with tuned MHC affinities and T-cell responses.

4. Andersson I.E., **Batsalova T.**, Haag S., Dzhambazov B., Holmdahl R., Kihlberg J., Linusson A. 2011. (E)-Alkene and Ethylene Isosteres Substantially Alter the Hydrogen-Bonding Network in Class II MHC A^q/Glycopeptide Complexes and Affect T-Cell Recognition. J. Am. Chem. Soc., 133(36):14368-78. (2011 impact factor – 9.907)

Abstract: The structural basis for antigen presentation by class II major histocompatibility complex (MHC) proteins to $CD4^+$ T-cells is important for understanding and possibly treating autoimmune diseases. In the work described in this paper, (E)-alkene and ethylene amide-bond isosteres were used to investigate the effect of removing hydrogen-bonding possibilities from the CII259-270 glycopeptide, which is bound by the arthritis-associated murine A^q class II MHC protein. The isostere-modified glycopeptides showed varying and unexpectedly large losses of Aq binding that could be linked to the dynamics of the system. Molecular dynamics (MD) simulations revealed that the backbone of CII259-270 and the A^q protein are able to form up to 11 hydrogen bonds, but fewer than this number are present at any one time. Most of the strong hydrogen-bond interactions were formed by the N-terminal part of the glycopeptide, i.e., in the region where the isosteric replacements were made. The structural dynamics also revealed that hydrogen bonds were strongly coupled to each other; the loss of one hydrogen-bond interaction had a profound effect on the entire hydrogenbonding network. The A^q binding data revealed that an ethylene isostere glycopeptide unexpectedly bound more strongly to Aq than the corresponding (E)-alkene, which is in contrast to the trend observed for the other isosteres. Analysis of the MD trajectories revealed that the complex conformation of this ethylene isostere was structurally different and had an altered molecular interaction pattern compared to the other A^q/glycopeptide complexes. The introduced amide-bond isosteres also affected the interactions of he glycopeptide/Aq complexes with T-cell receptors. The dynamic variation of the patterns and strengths of the hydrogen-bond interactions in the class II MHC system is of critical importance for the class II MHC/peptide/TCR signaling system.

5. Kostova Zh., **Batsalova T.**, Moten D., Teneva I., Dzhambazov B. 2015. Ragweed-allergic subjects have decreased serum levels of chemokines CCL2, CCL3, CCL4 and CCL5 out of the pollen season. Cent Eur J immunol, 40(4):442-446. (2015 impact factor – 0.309)

Abstract: CC-chemokines are important mediators of the allergic responses and regulate the cell trafficking.The aim of this study was to examine the serum levels of CCL2/MCP-1, CCL3/MIP-1α,

CCL4/MIP-1β and CCL5/RANTES , and to determine whether there are differences between ragweed-allergic subjects and healthy individuals out of the pollen season. Peripheral blood samples were collected from 24 subjects allergic to ragweed pollen and 12 healthy controls. Serum concentrations of chemokines/cytokines were measured by an enzyme-linked immunosorbent assay. We observed significantly decreased concentrations of CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β and CCL5/RANTES in the sera of ragweed-allergic patients compared to the healthy individuals (32.2 vs. 106.4 pg/ml, 89.5 vs. 135.7 pg/ml, 63.4 vs. 119.2 pg/ml and 11.2 vs. 18.1 ng/ml, respectively, $p < 0.01$). In contrast to the CC-chemokines, the serum levels of IL-8/CXCL8 showed a significant increase ($p < 0.05$) in the allergic group compared to the non-allergic subjects. Interleukin 4 levels were similar in both groups. In the sera of allergic patients, we have also detected significantly elevated levels of ragweed-specific IgE and IgG. However, decreased serum concentrations of the four CC-chemokines and elevated levels of IL-8/CXCL8 can be used as biomarkers for more accurate evaluation of the allergic status of patients with pollen allergy out of the season, to study the mechanisms for activation/inhibition of the subclinical allergic responses and for development of therapeutic strategies.

6. Zhelev I., **Batsalova T.**, Georgieva L., Dzhambazov B., Stoyanova A., Dimitrova-Dyugerova I. 2016. Chemical composition, cytotoxicity and antioxidant activity of essential oil from *Vitex agnus-castus* fruits, growing in Bulgaria. Oxidation Communications 39, 1-I: 145-156. (2015/2016 impact factor – 0.489)

Abstract: Fruit essential oils from *Vitex agnus-castus* L. growing in Bulgaria were obtained by water distillation and were analysed by gas chromatography. Thirty-five components were identified in a sample isolated from fruit material from Southern Bulgaria, with 1% oil content and main constituents (up to 3%): 1.8-cineole (20.39%), α-pinene (15.12%), β-pinene (9.40%), (Z)-β-farnesene (6.88%), bicyclogermacrene (6.08%), β-caryophyllene (5.27%) and terpinyl acetate (4.13%). Thirty-three components were identified in the essential oil sample from Northern Bulgaria, with 0.5% oil and main constituents: (Z)-β-farnesene (16.38%), bicyclogermacrene (12.26%), limonene (7.51%), α-pinene (6.24%), germacrene (4.12%), eicosane (4.03%), heneicosane (3.97%), β-pinene (3.99 %) and nonadecane (3.66%). The cytotoxicity of the essential oil from Southern Bulgaria against three human adenocarcinoma cell line LS180, cervical adenocarcinoma cells, lung adenocarcinoma cell lines (LS180, HeLa, A549) as well as normal amniotic cell line were investigated. V. agnus-castus fruit essential oil exhibited cytotoxic effects against all four tested cell lines. HeLa cell line showed the strongest sensitivity, suggesting a potential use of *Vitex agnus-castus* fruit essential oil as a chemotherapy agent for cervical cancer. The essential oil antioxidant activity measured by ABTS assay showed better values than those obtained with the DPPH assay.

7. Georgiev Y. N., Paulsen B. S., Kiyohara H., Ciz M., Ognyanov M. H., Vasicek O., Rise F., Denev P. N., Lojek A., **Batsalova T. G.**, Dzhambazov B. M., Yamada H., Lund R., Barsett H., Krastanov A. I., Yanakieva I. Z., Kratchanova M. G. 2017. *Tilia tomentosa* pectins exhibit dual mode of action on phagocytes as β -glucuronic acid monomers are abundant in their rhamnogalacturonans I. Carbohydr Polym., 175:178-191. doi: 10.1016/j.carbpol.2017.07.073. (2017/2018 impact factor - 5.158)

Abstract: Silver linden flowers contain different pectins (PSI-PSIII) with immunomodulating properties. PSI is alow-esterified pectic polysaccharide with predominant homogalacturonan region, followed by rhamno-galacturonan I (RGI) with arabinogalactan II and RGII (traces) domains. PSII and PSIII are unusualglucuronidated RGI polymers. PSIII is a unique high molecular weight RGI, having almost completely O-3glucuronidated GalA units with >30% O-3 acetylation at the Rha units. Linden pectins induced reactiveoxygen species (ROS) and NO generation from non-stimulated whole blood phagocytes and macrophages,resp., but suppressed OZP-(opsonized zymosan particles)-activated ROS generation, LPS-induced iNOSexpression and NO production. This dual mode of action suggests their anti-inflammatory activity, whichis known for silver linden extracts. PSI expressed the highest complement fixation and macrophage-stimulating activities and was active on intestinal Peyer's patch cells. PSIII was active on non-stimulatedneutrophils, as it induced ß2-integrin expression, revealing that acetylated and highly glucuronidatedRGI exhibits immunomodulating properties via phagocytes.

8. Georgiev Y. N., Ognyanov M. H., Kiyohara H., **Batsalova T. G.**, Dzhambazov B. M., Ciz M., Denev P. N., Yamada H., Paulsen B. S., Vasicek O., Lojek A., Barsett H., Antonova D., Kratchanova M. G. 2017. Acidic polysaccharide complexes from purslane, silver linden and lavender stimulate Peyer's patch immune cells through innate and adaptive mechanisms. Int J Biol Macromol., 105 (Pt 1):730-740. doi: 10.1016/j.ijbiomac.2017.07.095. (2017/2018 impact factor - 3.909)

Abstract: Three polysaccharide complexes (PSCs) were isolated from the aerial parts of common purslane (*Portulaca oleracea* L.), and the flowers of common lavender (*Lavandula angustifolia* Mill.) and silver linden (*Tilia tomentosa* Moench) by boiling water extraction and ethanol precipitation. The chemical composi-tion and immunomodulating effects of isolated PSCs were characterized. The chemical characterizationrevealed that the three samples contain mainly pectic polysaccharides. They exhibited ex vivo intestinalimmunomodulating activity through the murine Peyer's patch-mediated bone marrow cell proliferationtest at 100 g/ml concentration. At the same time, they stimulated ex vivo human blood T-cell popula-tions ($CD4^+/CD25^+$ and $CD8^+/CD25^+$), phagocytic leukocytes ($CD14^+$and $CD64^+$cells) and induced IL-6production from human white blood cells and Peyer's patch cells. The herbal PSCs stimulated ex vivo ROSproduction from whole blood phagocytes and showed unspecific in vitro anti-proliferative activity againstnormal and A549, HeLa and LS180 tumor cells. This is the first report on immunomodulating studies oflinden flower pectins and chemical and biological activity characterization of lavender polysaccharides.Our study demonstrates that similarly to purslane, lavender and silver linden herbal materials containimmunomodulating polysaccharides that could be useful for support of compromised immune system.

9. **Batsalova T.**, Bardarov K., Bardarov V., Moten D., Dzhambazov B. 2017. Cytotoxic properties of *Clinopodium vulgare* L. Extracts on selected human cell lines. *Comptes Rendus de L'Academie Bulgare des Sciences, 70 (5): 645-650*. (2017 impact factor – 0,27)

Abstract: Plants of *Lamiaceae* family are highly recommended as a source for new pharmaceuticals due to their wide range of biological activities. We have further investigated the in vitro effects of

acidified, alkalized and lipophilic extracts of the medicinal plant *Clinopodium vulgare* L. on the cell viability of selected cell lines. *In vitro* cytotoxicity was evaluated against CaOV (human testis cystadenocarcinoma), HeLa (human cervical adenocarcinoma), HT-29 (human colorectal adenocarcinoma) and FL (human amnion normal) cell lines using MTT assay. We found that two of the extracts (acidified and lipophilic) exerted selective dose-dependent cytotoxic activity against CaOV (IC50: 225–260.86 μg/mL) and HeLa cells (IC50: 360.27-388.5 μg/mL), while the cytotoxicity of the alkalized extract against these cell lines was less pronounced. All tested extracts showed very weak or lack of cytotoxic actions towards HT-29 and the normal FL cells. Moreover, these results indicate that *Clinopodium vulgare* extracts possess selective anticancer activity and could serve as a source for isolation and development of new therapeutic anticancer agents.

10. Teneva I., Klaczkowska D., **Batsalova T.**, Kostova Z., Dzhambazov B. 2016. Influence of captopril on the cellular uptake and toxic potential of microcystin-LR in non-hepatic adhesive cell lines. *Toxicon, 111: 50-57.* (2015 impact factor – 2.309)

Abstract: Microcystin-LR (MC-LR) is a toxin produced by various cyanobacterial strains. Its cytotoxicity is due to inhibition of the protein phosphatases PP1 and PP2A, resulting in hyperphosphorylation of a number of functional and cytoskeletal proteins. To penetrate through the plasma membrane, MC-LR needs specific transporters such the organic anion transporting polypeptides (OATP) that are highly expressed on the hepatocytes. Hence, our goal was to investigate the role of the membrane transport proteins for the cytotoxic effect of MC-LR on adhesive cell lines different from hepatocytes. We have used three cell lines - A549 (human lung carcinoma), SK-Hep-1 (human liver adenocarcinoma), FL (human amniotic normal cells), and two inhibitors of the OATP (cyclosporine A and captopril). To examine the cytotoxic effect of MC-LR we applied MTT and Neutral Red assays. In addition, a fluorescent staining of the mitochondria by JC-1 was performed. A dose-dependent cytotoxic effect was observed for the three cell lines, as this effect was most pronounced in A549. No cytotoxicity was detected when the captopril was added 2 h before treatment of the cells with MC-LR. Addition of captopril to the cells 2 h after treatment with MC-LR leads to enhancement of the cytotoxic effect. Reduced mitochondrial membrane potential after treatment with MC-LR was detected in the three cell lines, compared to untreated control cells. Results from the NRcytotoxicity assay indicated that MC-LR does not affect the lysosomes. Captopril is an effective inhibitor of both OATP influx membrane transport proteins and the P-gp efflux pumps involved in the transport of MC-LR. It protects the cells from toxic effects of the cyanotoxin MC-LR.

11. Ivanova A., Mikhova B., **Batsalova T.**, Dzhambazov B., Kostova I. 2011. New furostanol saponins from *Smilax aspera* L. and their in vitro cytotoxicity. *Fitoterapia, 82(2): 282-287.* (2011 impact factor – 1.848)

Abstract: The occurrence of the two new cis-fused A/B rings furostanol saponins (25S)-26-O-β-Dglucopyranosyl-5β-furostan-1β,3β,22α,26-tetraol-1-O-β-D-glucopyranoside and (25S)-26-O-β-D-glucopyranosyl-5β-furostan-1β,2β,3β,5β,22α,26-hexaol and the known compounds (25S)-26-O-β-D-glucopyranosyl-5β-furostan-3β,22α,26-triol-3-O-α-L-rhamnopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→2)-O-β-D-glucopyranoside and (25S)-26-O-β-D-glucopyranosyl-5β-furostan-

3β,22α,26-triol-3-O-β-D-glucopyranosyl-(1→2)-O-β-D-glucopyranoside, trans-resveratrol, (+) catechin and (−) epicatechin in the rhizomes of *Smilax aspera* is reported. All saponins have been isolated as their 22-OMe derivatives, which were further subjected to extensive spectroscopic analysis. The isolated furostanol saponins were evaluated for cytotoxic activity against human normal amniotic and human lung carcinoma cell lines using neutral red and MTT assays. In vitro experiments showed significant cytotoxicity in a dose dependent manner with IC50 values in the range of 32.98–94.53 μM.

12. Moten D., **Batsalova T.**, Basheva D., Mladenov R., Dzhambazov B., Teneva I. 2018. Outer membrane efflux protein (OMEP) is a suitable molecular marker for resolving the phylogeny and taxonomic status of closely related cyanobacteria. *Phycological research, 66 (1): 31-36.* DOI: 10.1111/pre.12203. (2017/2018 impact factor – 1,275)

Abstract: Taxonomy of *Cyanobacteria*, the oldest phototrophic prokaryotes, is problematic for many years due to their simple morphology, high variability and adaptability to diverse ecological niches. After introduction of the polyphasic approach, which is based on the combination of several criteria (molecular sequencing, morphological and ecological), the whole classification system of these organisms is subject to reorganization. The aim of this study was to evaluate whether the outer membrane efflux protein (OMEP) sequences can be used as a molecular marker for resolving the phylogeny and taxonomic status of closely related cyanobacteria. We have performed phylogenetic analyses based on the amino acid sequences of the OMEP and the DNA sequences of the 16S rRNA gene from 86 cyanobacterial species/strains with completely sequenced genomes. Phylogenetic trees based on the OMEP showed that most of the cyanobacterial species/strains belonging to different genera are clustered in separate clades supported by high bootstrap values. Comparing the OMEP trees with the 16S rDNA tree clearly showed that the OMEP is more suitable marker in resolving phylogenetic relationships within *Cyanobacteri*a at generic and species level.

13. **Batsalova T.**, Basheva D., Bardarov K., Bardarov V., Dzhambazov B., Teneva I. 2019. Assessment of the cytotoxicity, antioxidant activity and chemical composition of extracts from the cyanobacterium *Fischerella major* Gomont. Chemosphere, 218: 93-103. DOI: 10.1016/j.chemosphere.2018.11.097. (2017/2018 impact factor - 4.427)

Abstract: Cyanoprokaryotes (*Cyanobacteria/Cyanophyta*) are ancient photosynthetic prokaryotic organisms with cosmopolitan distribution. They are producers of a number of biologically active substances with antitumor and antifungal activity, vitamins, antibiotics, algaecides, insecticides, repellents, hormones, immunosuppressants and toxins. So far, the cyanobacterium *Fischerella major* Gomont has not been studied regarding its impact on the environment and human health. In this study, the cytotoxic, antioxidant and antitumor activities of four extracts prepared from *Fischerella major* were evaluated *in vitro*. In addition, the total phenolic content and the potential for production of cyanotoxins were also analyzed. The conducted GC/MS analysis identified 45 compounds with different chemical nature and biological activity. Presence of microcystins and saxitoxins was detected in all *Fischerella major* extracts. *In vitro* testing on cell cultures showed a significant concentration- and time-dependent cytotoxic effect on all cell lines (HeLa, SK-Hep-1 and FL) treated at three exposure times (24, 48 and 72 h) with four extracts. A selective antitumor effect was not

observed. This is the first study demonstrating biological activity of extracts from *Fischerella major*, which makes it an interesting subject for further research, including environmental risk assessments (as producer of cyanotoxins) or as a potential source of pharmaceuticals.

14. Sredkova P., **Batsalova T.**, Moten D., Dzhambazov B. 2020. Prebiotics can change the immunomodulatory properties of the probiotics. CEJI-00817-2017-02 (accepted for publication) – decision letter 103111. (2019/2020 impact factor – 1.455)

Abstract: The beneficial effects of the probiotics and prebiotics are mainly related to modulation of the composition and activities of the gut microbiota, and manipulating the immunological reactivity in autoimmune diseases. In the present study, we examined whether metabolic products from different strains of *Lactobacillus brevis* cultured with different prebiotics have similar immunomodulating properties on the immune cells under normal and inflammatory conditions using mouse model of collagen-induced arthritis (CIA). Two strains of *Lactobacillus brevis* (3448 and 8429) were cultured with four different prebiotics (xylooligosaccharides, inulin, pectin, chitosan). The sterile supernatants containing different metabolic products have been used for direct treatment of cell cultures prepared from CII-immunized mice and non-immunized (control mice). Our results showed that metabolic products from XOS decreased the levels of IFN-γ, IL-6, IL-17 and TNF-α in both cultures, from immunized and non-immunized mice. In contrast, the metabolic products from inulin, pectin and chitosan increased the concentrations of these cytokines with highest values for the pectin. Neither of investigated prebiotics influenced the secretion of IL-10. In addition, we found changes in the percentage of macrophages, which were different for the tested prebiotics. Also, the metabolic products from pectin and chitosan caused loss of T-cells ($CD3^+$) and increased percentages of $CD4^+CD25^+$ regulatory T cells and $CD8^+CD279^+$ anergic T cells. Hence, our data indicate that the immunomodulating properties of the probiotics are strain-specific and prebiotic-dependent.

SCIENTIFIC PUBLICATIONS IN JOURNALS WITHOUT IMPACT FACTOR

15. **Batsalova T.**, Ivanova P., Antova P., Dzhambazov B. 2016. Humoral autoimmune response against specific collagen type II epitopes in Bulgarian patients with rheumatoid arthritis. J BioSci Biotechnol. 5(1):45-52.

Abstract: Collagen type II (CII) is a strong candidate autoantigen for rheumatoid arthritis (RA) pathogenesis. CII is the main structural protein of synovial cartilage and it is attacked by both antibodies and T-cells during RA disease course. Experiments with mouse models have identified an immunodominant T-cell epitope from CII as well as several epitopes that are recognized by the majority of CII-specific autoantibodies. It has been shown that some epitope-specific anti-CII antibodies are arthritogenic and are associated with development of chronic arthritis. In addition, the immunodominant CII epitopes could be posttranslationally modified and these modified epitopes might be involved in induction and/or perpetuation of autoimmune humoral response and arthritic pathology. The aim of the present study was to evaluate the CII epitope-specific humoral response in a subgroup of Bulgarian patients with rheumatoid arthritis. Our results demonstrate that RA patients have significantly increased levels of anti-CII antibodies compared to healthy individuals and patients with other type of autoimmune disease. The majority of anti-CII antibodies in Bulgarian

patients are directed against the U1 and J1 conserved epitopes. We show that D8 epitope-specific antibodies react to the triple-helical structure of the epitope and thus recognize both the native and the posttranslationally citrullinated D8. This is the first article presenting an evaluation of CII-specific humoral autoimmune response in Bulgarian patients with rheumatoid arthritis.

16. **Batsalova T.**, Kostova Z., Moten D., Teneva I., Dzhambazov B. 2017. Serum levels of certain CC and CXC chemokines in birch pollen allergic individuals out of the pollen season. Advances in Biology & Earth Sciences, Vol.2, No.1, p. 22-33.

Abstract: Chemokines play a key role in the regulation of cell trafficking during immune responses. In pollen-allergic individuals, the roles of chemokines have been predominantly studied during active allergic reactions. However, little is known about chemokine levels and their effect on immune responses out of the pollen season when atopic individuals do not show clinical symptoms of allergy. Therefore, the aim of the present study was to investigate the serum levels of CCL11/Eotaxin, CCL17/TARC, CCL22/MDC, CXCL1/GROα, CXCL9/MIG, CXCL10/IP-10, and CXCL11/I-TAC out of the pollen season and to determine whether there are differences between birch pollen-allergic and non-allergic individuals. We observed significantly increased concentrations of CCL11/Eotaxin ($p<0.01$), CCL17/TARC ($p<0.01$), CCL22/MDC ($p<0.01$), CXCL9/MIG ($p<0.05$) and CXCL10/IP-10 ($p<0.05$) in the sera of birch pollen-allergic patients compared to healthy individuals. In contrast, the serum levels of CXCL1/GROα were lower ($p<0.05$) in the allergic group compared to the non-allergic subjects. Furthermore, IFN-γ and IL-17 levels were significantly elevated ($p<0.05$) in the sera of birch-pollen allergic individuals. These results suggest persistent Th17 activity in birch pollen-allergic individuals. The detected differences implicate a role of these chemokines in subclinical allergic responses, which could provide the basis for development of new therapies and strategies for disease monitoring.

17. Sredkova P., Iliev I., Dzhambazov B., **Batsalova T.** 2017. Prebiotic Treatment Influence the Adhesion Properties of Three *Lactobacillus* strains. Int.J.Curr.Microbiol.App.Sci, 6(9): 2914-2924.

Abstract: Adhesion to intestinal epithelial cells is commonly analyzed during investigations on potential probiotic activity of certain bacteria. The aim of the present study was to investigate whether different prebiotics affect the adhesion properties of three strains lactobacilli (*Lactobacillus rhamnosus* 1010, *Lactobacillus acidophilus* 11, and *Lactobacillus paracasei* 8458). Using in vitro model system based on cell lines derived from colon epithelial cells, we have determined the adherence ability of the three bacterial strains following treatment with 6 different prebiotics (inulin, pectin, chitosan, galactooligosaccharides, xylooligosaccharides and beta-glucan). Our results demonstrated significant reduction of lactobacilli adhesion after treatment with beta-glucan and chitosan. Conversely, xylooligosaccharides- and galacto-oligosaccharides-treated bacteria showed enhanced ability to adhere to enterocyte-like cell lines. Treatment with inulin did not show pronounced effect on lactobacilli adhesion. Overall, the three tested *Lactobacillus* strains displayed similar responses to the prebiotic treatment. Only pectin induced strain specific effect – reduced adhesion of *L. rhamnosus* 1010 and *L. acidophilus* 11 while the adhesion properties of *L. paracasei* 8458 were not significantly affected. These findings suggest that different types of prebiotic

substances induce different mechanisms that modulate the adhesion ability and probably the probiotic activity of lactobacilli.

SCIENTIFIC PUBLICATIONS IN CONFERENCE AND CONGRESS PROCEEDINGS

18. **Batsalova T.**, Moten D., Mateev B., Dzhambazov B. 2018. Effects of iron oxide (II, III) nanoparticles exposure: *in vitro* evaluation using algal and human cells. 18th International multidisciplinary scientific geoconference SGEM 2018, Conference proceedings, vol. 18, issue 6.1, pp. 177-183. DOI: 10.5593/sgem2018/6.1/S24.024. (SJR 0.21)

Abstract: Iron oxide (II, III) nanoparticles (ION) are used in variety of biomedical and industrial applications (for instance, in gene therapy, cell tracking, magnetic resonance imaging, drug delivery therapies, in vitro diagnostic assays, catalytic materials, wastewater treatment adsorbents, nanoadjuvants for vaccine and antibody production, coatings, gas sensors, ion exchangers, magnetic recording devices, magnetic data storage devices). In relation to their vast utilization there is a need for more information on the effects of ION exposure. Therefore, the present study aimed to investigate the influence of various concentrations of iron oxide (II, III) nanoparticles *in vitro* using different types of cells - three algal strains (*Dunaliella salina*, *Chlamydomonas asymmetrica*, *Scenedesmus obtusiusculus*) and three human cell lines (A549, HeLa, normal dermal fibroblasts). The cells were incubated on 96-well culture plates in growth medium containing nanoparticles for 15 h and 72 h. The cytotoxicity of ION was determined by the MTT assay. The obtained results demonstrated significantly reduced mammalian and algal cell growth after 72 h treatment with ION. Cellular ATP production was diminished following exposure to ION. In addition, presence of nanoparticles in the culture medium resulted in decreased mobility of *Dunaliella salina* and *Chlamydomonas asymmetrica*. ION exposure induced higher total protein production in all types of algal cells, which indicates activation of specific reaction against nanoparticle induced toxicity. Further experiments are needed to determine this mechanism. Our results demonstrate significant effects of ION exposure in both mammalian and algal cells.

19. **Batsalova T.**, Moten D., Mateev B., Dzhambazov B. 2018. Biofunctionalized nanoparticles as diagnostic tools for autoimmune diseases. 18th International multidisciplinary scientific geoconference SGEM 2018, July 2-8, 2018. Conference proceedings, vol. 18, issue 6.1, pp. 83-89. DOI: 10.5593/sgem2018/6.1/S24.012. (SJR 0.21)

Abstract: Autoimmune diseases affect approximately 5% of the population worldwide. Generally, these disorders induce high degree of morbidity and disability and therefore, they have a significant social and economic impact. The precise diagnosis of specific autoimmune response is necessary for adequate treatment of the patient. Accordingly, the diagnostic assays for autoimmune diseases are continuously developing and improving. Biofunctionalized nanoparticles represent a promising novel approach in this field. Our group has developed a mechanism for efficient biofunctionalization of iron oxide (II, III) nanoparticles (ION) with molecules used for detection of biomarkers specific for rheumatoid arthritis. Purified biofunctionalized ION were tested with serum samples from healthy individuals, patients with rheumatoid arthritis and patients with another autoimmune disorder. We were able to detect rheumatoid arthritis-specific biomarkers using biofunctionalized ION. The

efficiency of the nanoparticle-based assay was similar to the conventional enzyme-linked immunosorbent assay. Our results provide a basis for development of new utility models for rapid and sensitive diagnostic assays for autoimmune diseases.

TEXTBOOKS

20. Dzhambazov B., **Batsalova T.** 2014. Practical classes in Cell biology (second supplemented and revised edition), 94 pages. University publishing house „Paisii Hilendarski“. ISBN 978-954-423-969-5.

This textbook is intended for use by students majoring in "Biology" and "Medical Biology" at Plovdiv University "Paisii Hilendarski", but it can also be used by students majoring in various biological fields and related specialties from other universities. It reveals theoretically and demonstrates in practice the basic structural elements of the cell and their functions.

21. **Batsalova T.**, Moten D., Dzhambazov B. 2020. Guide for laboratory classes in Animal Cell Cultures, 88 pages. University publishing house „Paisii Hilendarski“. ISBN 978-619-202-590-8.

The textbook can be used for practical classes in the disciplines Animal *in vitro* cultures, Cell and tissue cultures, Animal cell cultures and hybridoma technologies, Stem cells and regeneration, as well as in other biological disciplines, including the application of techniques for *in vitro* culture of animal and human cells. It presents methods for maintaining sterility when working with *in vitro* cultures, as well as basic techniques for establishing cell lines, methods for manipulating and maintaining animal and human cells in culture.

II Self-evaluation of scientific contributions

The contributions of the submitted materials for participation in the competition for the academic position "Associate Professor" in the scientific specialty of Cell Biology can be grouped in several areas:

1) Contributions to the analysis of the autoimmune responses and elucidation of the cellular-molecular mechanisms of autoimmune diseases.
2) Contributions towards the field of *in vitro* cytotoxicology and analysis of biological activity.
3) Contributions towards elucidation of the role of different types of chemokines in pollen allergies out of the pollen season.
4) Contributions to the resolution of the taxonomic status of cyanobacterial species.
5) Contributions towards evaliating the effect of prebiotics on the immunomodulatory and adhesion properties of different species and strains of the genus *Lactobacillus*.

6) Contributions in the field of bionanotechnology - *in vitro* analyzes of the cytotoxicity of iron oxide nanoparticles; biofunctionalization of nanoparticles and development of methods for diagnosis of autoimmune diseases.

7) Contributions towards the field of education.

1) Contributions to the analysis of the autoimmune responses and elucidation of the cellular-molecular mechanisms of autoimmune diseases.

The immune system protects the body from pathogens through its unique ability to distinguish between its own and foreign structural components. This ability is based on complex interactions in which T-lymphocytes play a key role in activating or not activating immune responses. The presented scientific publications on this issue include elucidation of the cellular-molecular mechanisms in the autoimmune disease rheumatoid arthritis and the development of methods and means for treatment and diagnosis of this disease.

Rheumatoid arthritis is a systemic autoimmune disease, generally caused by a violation of tolerance-building mechanisms. As a result, the amount of autoreactive T cells increases, attacking mainly the joints, as well as other parts of the body.

Collagen type II (CII) is considered to be a major candidate autoantigen in the pathogenesis of rheumatoid arthritis (RA). It is a major structural protein of articular cartilage. During the development of RA, CII is attacked by antibodies and T cells. Experiments with mouse models identified an immunodominant T-cell epitope from CII and several B-cell epitopes recognized by most CII-specific antibodies. Some epitope-specific anti-CII antibodies have been shown to be arthritogenic and to play a role in the development of chronic arthritis. On the other hand, the immunodominant T-cell CII epitope can be posttranslationally modified and it is known that its modified forms could be involved in the induction and/or maintenance of the autoimmune humoral response and arthritic pathology. For example, glycosylated lysine at position 264 in this epitope has been shown to be central to T-cell recognition and induction of immunological tolerance to CII. In addition, the Ncf1 gene, which encodes one of the subunits of the phagocytic NADPH oxidase complex NOX2, plays an important role in immunological tolerance to CII.

Publication № 1 presents a study of the effect of oxidation on the efficacy of immunespecific vaccination with complexes of MHC class II molecule and glycosylated CII peptide. Our experiments showed that normal levels of reactive oxygen species (ROS) contribute to the establishment of immunological tolerance and prevent the development of arthritis, as only murine models with a functional Ncf1 gene do not develop collagen-induced arthritis (CIA) after vaccination with glycolsylated CII259-273 peptide and MHC class II molecule. Transfer of T cells from vaccinated mice with functional Ncf1 protein resulted in suppression of clinical symptoms of arthritis in B10.Q mice, whereas in a mutant Ncf1 mouse line (Ncf1 $^{*/*}$) a significantly weaker effect was observed, which clarifies that ROS modify the secondary rather than the primary immune response. In addition, the publication demonstrates that during the primary response to vaccination with glycosylated CII259-273 peptide and MHC class II molecules regulatory T cells, elevated levels of negative costimulant molecules and anti-inflammatory cytokines are present in both Ncf1 mutant mice and wild-type B10.Q mice which explains the observed ROS-independent vaccination effect.

The results obtained in these studies may find application in the treatment of patients with rheumatoid arthritis, as the glycosylated form of the peptide CII259-273 binds to human MHC II molecules (DR1 and DR4). In addition, T cells specifically responsive to this peptide have been identified in patients with rheumatoid arthritis.

In order to increase the sensitivity of existing "humanized" mouse models for the study of rheumatoid arthritis in humans, a new "humanized" mouse model (strain B10.DR4.Ncf.1$^{*/*}$) was created by introducing a gene (Ncf.1$^{*/*}$) enhancing susceptibility to autoimmune diseases (**Publication № 2**). A comparative analysis was performed showing that C1-specific anti-CII antibodies dominated the A^{q}-expressing model B10.Q compared to antibodies against the other two CII B-cell epitopes (U1, J1). The humoral response in the B10.DR4.Ncf.1$^{*/*}$ model is dominated by C1- and U1-specific anti-CII antibodies. The unmodified CII259-273 epitope of type II collagen was found to play a major role in activating CII-specific pathogenic T cells in the development of collagen-induced arthritis when the *in vivo* expressed MHC II haplotype is DR4. It was observed that the activation of DR4-restricted CII-specific T cells depend mainly on the lysine residue at position 264 of the CII259-273 epitope, which is recognized in both unmodified and hydroxylated forms.

Posttranslational modifications of proteins are extremely important for the induction and pathogenesis of a number of autoimmune diseases, including RA. The contribution of **Publication № 3** is related to the development of a methodology for studying the interactions between glycosylated peptides and receptors based on a combination of structural-based virtual screening, ligand-based statistical molecular design and biological research. The methodology involves the design of a CII259-273 glycopeptide library, in which the two key positions that are crucial for binding to A^{q} and DR4 molecules are varied. The synthesis and biological evaluation of the designed glycopeptides make it possible to establish the structure-activity relationship (SAR) and to understand how they bind to A^{q} and DR4. Groups of glycopeptides with different responses have been selected. They are of great interest for conducting research related to collagen-induced arthritis with regard to the development of specific vaccines. The structure-activity relationship (SAR) established in this study provides a platform for the development of second-generation glycopeptides with a specific MHC II affinity and T-cell responses. This strategy can be applied for design of new ligands for any type of protein-ligand system if there is an established structural model.

Structural analysis of the antigens presented by MHC class II proteins is extremely important for clarifying the pathogenesis and the possibilities for treatment of RA. **Publication № 4** used (E) - alken- and ethylene-amide-linked isosteres to investigate the effect of eliminating the hydrogen bonding potential of the CII259-270 glycopeptide that binds to murine A^{q} MHC class II molecules durring development of CIA. It was found that the dynamic change in the ways and strength of interaction between CII259-270 and MHC class II is critical for the signaling system MHC class II - peptide - T-cell receptor.

An analysis of the CII-specific humoral immune response in Bulgarian patients with rheumatoid arthritis was published for the first time in **Article №15**. Significantly elevated levels of anti-CII antibodies have been demonstrated in RA patients compared to healthy individuals and patients with other types of autoimmune disease. It has been clarified that the predominant part of the anti-CII antibodies in Bulgarian RA patients show specificity for the U1 and J1 immunodominant B-

cell epitopes. At the same time, recognition of the triple-helical structure of the D8 epitope and the presence of reactivity to both its native and post-translational citrullinated form was detected.

2) **Contributions towards the field of *in vitro* cytotoxicology and analysis of biological activity.**

Clarification of the cytotoxicity and biological activity of extracts, essential oils and purified new molecules from various plant species and microalgae is progressively developing scientific field with high potential for identifying new candidate agents and establishing nutritional supplements with beneficial effects on the immune system and general state of the organism. **Publications № 6, 7, 8, 9, 11, 13** contribute to this field.

Article №6 defines the composition of essential oils isolated from the fruits of the plant *Vitex agnus-castus* L, developing in Southern and Northern Bulgaria. The antioxidant activity of the essential oil from Southern Bulgaria and its *in vitro* cytotoxicity to human cell lines was studied for the first time. The highest level of toxicity was reported relative to the adenocarcinoma cell line HeLa, which stimulates further analyses of the test sample and purification of biologically active molecules for use in the treatment of cervical cancer.

Publication №7 proves insight to the immunomodulatory properties of pectins isolated from the flowers of silver linden (*Tilia tomentosa*). The chemical composition of three linden polysaccharides is characterized. Isolated pectins have been shown to induce reactive oxygen species (ROS) and NO production in unstimulated phagocytes, but inhibited the OZP (opsonized zymosan particles) -activated ROS production, LPS-induced iNOS expression, and NO production. This dual of action suggests for anti-inflammatory activity of the polysaccharides.

Publication №8 presents new knowledge on the chemical and biological activity of three polysaccharide samples (PPS) isolated from the aerial parts of common purslane (*Portulaca oleracea* L.), from lavender (*Lavandula angustifolia* Mill.) and silver linden (*Tilia tomentosa* Moench). The obtained results prove the immunomodulatory properties of the studied polysaccharides and provide a perspective for the application of these substances in therapies that stimulate and support the activity of the immune system.

Plants of the *Lamiaceae* family are often used as a source of new pharmaceuticals due to their broad-spectrum biological activity. In this regard, **Publication №9** clarifies the *in vitro* effects on a panel of cell lines treated with acidified, alkalized or lipophilic extract of the medicinal plant *Clinopodium vulgare* L. The reported results show that the acidified and lipophilic extract of *Clinopodium vulgare* have selective anti-cancer activityagainst HeLa and CaOV cells. Therefore, these extracts can be used as a source of substances with therapeutic antitumor potential.

Steroid saponins have a number of biological activities such as hemolytic activity, antidiabetic, anticoagulant and antitumor activity, as well as the formation of cholesterol complexes. **Publication №11** examined the biological activity of two new furostanol saponins isolated from *Smilax aspera* (*Liliaceae*). The compounds were studied with *in vitro* cytotoxicity assays on human normal amniotic cells and human lung cancer cells. The results show significant cytotoxicity with dose-dependent effect and IC_{50} values in the range of 32.98-94.53 μM.

Microalgae, including *Cyanoprokaryota*, are known to be a rich source of biologically active peptides, macrolides, alkaloids, fungicides, and enzyme inhibitors for therapeutic use. Recently, more and more attention has been paid to cyanoprokaryotes as potential sources of pharmaceuticals with different structure and biological activity, including cytotoxicity, immunosuppression, antiproliferative activity, and antitumor activity.

Article №13 reports for the first time the biological activity of extracts of *Fischerella major* Gomont. An analysis of cytotoxic, antioxidant and antitumor activity, cyanotoxin content of four different extracts is presented. The data obtained identify *Fischerella major* as an object of interest for further research, including environmental risk assessment (as a producer of cyanotoxins) and the possibility of obtaining substances for pharmaceutical use.

Microcystin-LR (MC-LR) is a toxin that is synthesized by many cyanobacterial species. Its cytotoxicity is due to inhibition of PP1 and PP2A protein phosphatases, leading to hyperphosphorylation of a number of functional and cytoskeletal proteins. To enter the cell, MC-LR passes the plasma membrane through specific transporters such as the organic anion polipeptide transporter (OATP), which is actively expressed on the surface of hepatocytes. Captopril is an effective inhibitor of OATP *influx* membrane transport proteins as well as P-gp *efflux* pumps involved in the transport of MC-LR. **Publication №10** demonstrates for the first time the protective effect of captopril against the toxic potential of MC-LR.

3) Contributions towards elucidation of the role of different types of chemokines in pollen allergies out of the pollen season.

Pollen allergy is one of the most common allergies worldwide and it is associated with cross-reactivity of IgE. This type of allergy can also cause the development of hypersensitivity to plant foods, inducing primary sensitization of sensitive individuals, which in turn leads to cross-reactivity and secondary allergy to foods of plant origin.

Chemokines play a major role in the regulation of cellular trafficing during immune responses. In individuals with pollen allergy, the importance of chemokines has been studied mainly during active allergic reactions. Little is known about chemokine levels and their effect on the immune response out the pollen season, when allergic individuals show no clinical symptoms. Information on this scientific issue is provided in **Publication №5,** which presents an analysis of serum concentrations of CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β and CCL5 RANTES and IL-8/CXCL8 in patients allergic to ragweed. Decreased concentrations of CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β and CCL5 RANTES and elevated levels of IL-8/CXCL8 have been detected. Thus, these chemokines can be used as biomarkers for more accurate assessment of the allergic status of patients with pollen allergy out of season, to study the mechanisms of activation/inhibition of subclinical responses and the development of therapeutic strategies.

Publication №16 presents a study of serum levels of CCL11/Eotaxin, CCL17/TARC, CCL22/MDC, CXCL1/GROα, CXCL9/MIG, CXCL10/IP-10 and CXCL11/I-TAC in individuals allergic to birch pollen outside the pollen season . Significantly elevated concentrations of CCL11/Eotaxin ($p < 0.01$), CCL17/TARC ($p < 0.01$), CCL22/MDC ($p < 0.01$), CXCL9/MIG ($p < 0.05$) and CXCL10/IP-10 were measured ($p < 0.05$) and Th17 activity was detected in the sera of patients

with birch pollen allergy compared to healthy individuals. The obtained data provide a basis for the development of new therapies and strategies for monitoring pollen allergy.

4) **Contributions to the resolution of the taxonomic status of cyanobacterial species.**

For a long time, the taxonomy of cyanobacteria has been a significant scientific problem due to their simple morphology, high variability and adaptability to a variety of ecological niches. After the introduction of the polyphasic approach, based on a combination of several criteria (molecular research /sequencing/, morphological and ecological criteria), the whole classification system of these organisms became an object of reorganization. With regard to this scientific issue, **Publication №12** contributes to the elucidation of new taxonomic biomarkers based on the polyphasic approach. The paper presents phylogenetic analyzes based on the amino acid sequences of the outer membrane efflux protein (OMER) and DNA sequences of the 16S rRNA gene of 86 cyanobacterial species with fully sequenced genomes and proves that OMEP is a more appropriate marker for resolving the taxonomic status of different cyanobacteria.

5) **Contributions towards evaliating the effect of prebiotics on the immunomodulatory and adhesion properties of different species and strains of the genus *Lactobacillus*.**

The positive effects of probiotics and prebiotics are mainly due to modulation of the composition and activities of the intestinal microbiota, as well as modulation of immunological reactivity in autoimmune diseases. **Publication №14** proves that the immunomodulatory properties of probiotic bacterial strains are species-specific and prebiotic-dependent. New information is provided on the role of metabolic products from *Lactobacillus brevis* strains cultured in the presence of different prebiotics. Metabolic products from xylooligosaccharides decreased the levels of IFN-γ, IL-6, IL-17 and TNF-α in cell cultures from immunized mice and in control leukocyte cultures (derived from nonimmunized mice). In contrast, the metabolic products of inulin, pectin and chitosan increased the production of these cytokines. IL-10 secretion was not affected by the prebiotics studied. In addition, based on immunophenotypic analysis, a decrease in the total T cell population was demonstrated as a result of treatment with pectin and chitosan metabolic products, combined with an increase in the populations of $CD4^+$ $CD25^+$ regulatory T cells and $CD8^+$ $CD279^+$ anergic T - cells.

Adhesion to intestinal cells is a frequently analyzed parameter in studies that evaluate the probiotic potential of certain types of bacteria. In this regard, **Publication №17** contributes to elucidating the influence of different types of prebiotics on the adhesion abilities of three *Lactobacillus* species. Adverse effects on bacterial adhesion have been reported after treatment with beta-glucan and chitosan, while treatment with xylooligosaccharides and galactooligosaccharides improved the adhesion of lactobacilli to enterocyte-like cell lines.

6) **Contributions towards the field of bionanotechnology - *in vitro* analyzes of the cytotoxicity of iron oxide nanoparticles (ION); biofunctionalization of nanoparticles and development of methods for diagnosis of autoimmune diseases.**

Iron oxide (II, III) nanoparticles have wide biomedical and industrial applications (for instance, in the development of gene therapies, cell tracking techniques, magnetic resonance imaging, targeted drug delivery therapies, *in vitro* diagnostic tests, adsorbents for wastewater treatment, nanoadjuvants

for vaccine and antibody production, specific coatings, magnetic recorders, gas sensors, magnetic storage devices, etc.). Therefore, there is a need for more information on the potential toxic effects of ION. **Publication №18** defines the *in vitro* cytotoxicity of iron oxide (II, III) nanoparticles towards different types of human and microalgae cells. The results show significantly reduced cell growth and ATP production under the influence of ION treatment, as well as reduced motility of *Dunaliella salina* and *Chlamydomonas asymmetrica* cells. ION provoked higher total protein production in algal cells, suggesting activation of a specific response to nanoparticle-induced cytotoxicity. The results presented in this article define the cyto- and ecotoxicological potential of ION and prove the need for a more detailed study of their biological activity.

Autoimmune diseases affect approximately 3 to 5% of the population worldwide and represent significant social and economic burden for modern society, similar to that of cancer and cardiovascular disease. Accurate diagnosis of the specific autoimmune response is critical to adequate patient therapy. Therefore, researchers are actively working on improving and increasing the specificity of diagnostic tests for autoimmune diseases. A new prespective in this regard are the biofunctionalized nanoparticles. **Publication №19** contributes to the development of a new type of rapid and sensitive diagnostic tests for rheumatoid arthritis. A methodology for efficient biofunctionalization of iron oxide (II, III) nanoparticles with molecules used for detection of biomarkers for RA has been developed. The obtained modified nanoparticles specifically bind and detect the presence of RA diagnostic markers in serum samples.

7) Contributions towards the field of education.

Two textbooks for practical classes in Cell Biology and Animal Cell Cultures, respectively, are presented. They are intended for use by students from different biological specialties of Plovdiv University "Paisii Hilendarski". They are in accordance with the study plan of the students and the number of study hours, but can also be used by students in various biological fields and related specialties from other higher education institutions.

In the practical textbook in Cell Biology the basic structural elements of the cell and their functions are presented theoretically and demonstrated in practice. Classical and new methods for studying the cell structure and processes are presented.

The textbook on Animal Cell Cultures introduces students to the methods for maintaining sterility when working with *in vitro* cultures, as well as the basic techniques for establishing cell lines, methods for manipulating and maintaining animal and human cells in culture.

Plovdiv, Prepared by: …….

September, 2020 /ch. assist. prof. Tsvetelina Batsalova PhD/