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1 – 4 October 2011
Arandjelovac, Serbia

organized by:

Bernhard Fleischer, Miodrag Lukic, Hannes Stockinger, H. Joachim Seitz and Moncef Zouali

supported by:

PROGRAM

SATURDAY, 1 OCTOBER 2011

Registration **15.00**

Opening **17.00**

Plenary Lecture 1
Stipan Jonjic, University of Rijeka, Rijeka, Croatia **17.30**
Viral interference with activating and inhibitory NK receptors

Welcome reception, dinner **18.30**

SUNDAY, 2 OCTOBER 2011

Plenary Lecture 2 **09.15**
Hannes Stockinger, Medical University of Vienna, Vienna, Austria
Yin/Yang of adaptive immunity between cure and destruction

Plenary Lecture 3 **10.00**
Sergei Nedospasov, Engelhardt Institute of Molecular Biology, Moscow, Russia
Physiological function of TNF and promises/problems of anti-cytokine therapy

Coffee break **10.45**

Plenary Lecture 4 **11.15**
Tchavdar Vassilev, Bulgarian Academy of Sciences, Sofia, Bulgaria
Sepsis and experimental sepsis treatment

Plenary Lecture 5 **12.00**
Bernhard Fleischer, Bernhard Nocht Institute, Hamburg, Germany
Costimulation of T cells

Lunch break **12.45**

Plenary Lecture 6 **15.00**
Moncef Zouali, University Denis Diderot, Paris, France
Role of B cells in Autoimmunity

PROGRAM

Coffee break **15.45**

Free time **16.00**

Student's poster viewing and poster workshop with dinner buffet **19.00**

MONDAY, 3 OCTOBER 2011

Plenary Lecture 7 **09.15**

Seppo Meri, University of Helsinki, Helsinki, Finland

Microbial escape of innate immunity

Plenary Lecture 8 **10.00**

Gerold Stanek, Medical University of Vienna, Vienna, Austria

Tick-borne bacterial infections in Europe; diagnosis and management

Coffee break **10.45**

Plenary Lecture 9 **11.15**

Miodrag Lukic, University of Kragujevac, Kragujevac, Serbia

IL-33/ST2 signaling in immunopathology and immunity to tumors

Plenary Lecture 10 **12.00**

Annette Gospos, Euroimmun, Luebeck, Germany

Antigens for diagnostics of autoimmune diseases

Lunch break **13.00**

Parallel Workshops/Discussion groups **15.00**

Annette Gospos

Practical microscopy for autoantibodies (in groups of max. 12)

15:00 – 1st group

16:30 – 2nd group

17:30 – 3rd group

PROGRAM

Parallel sessions **15.00**

Bernhard Fleischer: **How to measure T cell responses**

Seppo Meri: **Complement activation and regulation**

Coffee break **16.00**

Gerold Stanek: **16.30**

Lyme borreliosis - the European perspective in diagnosis and management

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Hannes Stockinger: **17.30**

Novel ultra-sensitive live-cell imaging techniques for understanding immune cell functions

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Farewell dinner **18.15**

TUESDAY, 4 OCTOBER 2011

Plenary Lecture 11 **09.15**

Miodrag Stojkovic, University of Kragujevac, Kragujevac, Serbia

What came first? Egg or…?

Plenary Forum and Final Discussion **10.00**

H. Joachim Seitz, University of Hamburg, Hamburg, Germany

Hannes Stockinger, EFIS

Support for young scientists in Germany and Europe

Farewell, Coffee, Sandwiches, Certificates, Questionnaire…. **11.00**

Departure **12.00**

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IN VITRO DECIDUALIZATION OF ENDOMETRIAL STROMAL CELLS USED AS A PREDICTION FACTOR FOR SUCCESSFUL EMBRYO IMPLANTATION

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Introduction:

Pregnancy depends on close interactions between the embryo and the receptive endometrium during the process of implantation. The transient period of endometrial receptivity in humans coincides with differentiation of endometrial stromal cells (ESCs) into highly specialized decidual cells. The decidualization is characterized by differentiation of the endometrial stromal fibroblasts into secretory, epitheloid-like decidual cells and the influx of specialized uterine immune cells. The decidualized ESCs become rounded and secrete phenotypic antigens, including prolactin (PRL) and IGFBP-1.

There is no doubt that the impaired decidualization would have negative effect on the implantation. We test our hypothesis that the *in vitro* decidualization of ESCs could be used as a prediction factor for successful embryo implantation using quantitative comparison of secreted PRL and IGFBP-1.

One of the aims of this project is to study the mechanisms of modulation on basic immune functions such as secretion of immunoglobulins, cytokines, proliferation of T-cells. The objective is to compare the immunomodulatory activity of ESCs isolated from two groups of women- one with successful pregnancy and the other with unsuccessful one.

Materials an Methods:

Isolation and culture of ESCs

Samples of endometrium were obtained from women undergoing hysterectomy. The ESCs were isolated using enzyme digestion and plated in culture flasks in DMEM supplemented with 10% fetal bovine serum (FBS) and antibiotics.

In Vitro Decidualization

Primary cultures of ESCs were decidualized by administering cAMP and Medroxyprogesterone for 8 days. The culture medium used was DMEM supplemented with 2% charcoal-stripped FBS and antibiotics. The medium was changed every other day. The levels of IGFBP1 and prolactin were determined using ELISA and Real-Time PCR.

Results:

Our findings show that there is a correlation between the level of the endometrial decidualization and the rate of successful embryo implantations in women with repeated implantation failure. ESCs that secrete high levels of PRL are correlated with women with successful embryo implantation.

Conclusion:

Impaired decidualization may serve as a predictive factor for successful embryo implantation.

EFFECTS OF MITOXANTRONE TREATMENT ON THE EVOLUTION OF NK CELL PHENOTYPE OF MS PATIENTS

Coralie Chanvillard, M. Lozano, I. Hamann, F. Paul, J.M. Dorr, C. Infante-Duarte

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Multiple sclerosis (MS) is assumed to be an autoimmune disease initiated by autoreactive T cells that recognize central nervous system antigens. Although adaptive immunity is clearly involved in MS pathogenesis, innate immunity increasingly appears to be implicated in the disease. We and others have presented evidence that natural killer (NK) cells may be involved in immunoregulation in MS, leading to debate as whether a particular NK cell subtype will account for this effect.

In MS, several therapeutic approaches have been developed in order to reduce the risk of relapses and progression of patient disability. Immunomodulatory drugs, such as interferon-beta and glatiramer acetate, are commonly used for the relapsing-remitting form of the disease. However, these agents are ineffective in a substantial group of patients, in particular in patients with a chronic progressive disease course. Consequently, more aggressive treatments are currently used as escalation therapy for these patients. One of these drugs is mitoxantrone, a potent cytotoxic immunosuppressive treatment, which demonstrates efficacy for rapidly aggravating MS. Mitoxantrone targets principally proliferating T and B cells, and induces apoptosis of antigen-presenting cells. However, little is known about its effect on natural killer (NK) cells.

To monitor the impact of mitoxantrone on adaptive and innate immune cells, we initiated a clinical study in a cohort of patients with secondary-progressive MS treated intravenously with mitoxantrone for up to 18 months (12 mg/m2 infusion every 3 months). A longitudinal study was conducted comparing intra-individually (baseline vs. treatment) major populations of peripheral blood lymphocytes using flow cytometry. As previously shown, the B cell fraction was drastically reduced during treatment. However, the NK cell fraction increased significantly. To better understand how treatment influences NK cells, we have performed additional investigations of different NK cell markers including an array of NK cell receptors (KIR, NKG2A, NKp30 and NKp46) and maturation markers (CX3CR1, CD27, CD62L and CD57).
Here we present this longitudinal study of the evolution of NK cell subset distribution and phenotype alteration in MS patients following mitoxantrone treatment.

INVESTIGATION OF THE INTERACTIONS OF RECOMBINANT BANANA LECTIN WITH THE MUCOSAL SURFACE OF THE MOUSE DIGESTIVE TRACT

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Objective:

Banana lectin is a food protein which is being consumed by a vast number of people, with no noted harmful effects. In order to assess the potential usage of recombinant banana lectin as an immunostimulator it was of interest to analyze the effect it exerted *in vivo* in mice, upon two different routes of exposure: oral and intramuscular.

Methods:

In this study Western blot analysis of stomach and intestinal content was used, as well as Immunofluorescence detection of recombinant banana lectin in mice intestinum. Specific antibody titre was determined by use of ELISA

Results:

It was found that recombinant banana lectin is very stable *in vivo* in the mouse digestive tract, and that it specifically interacts with the mucosal surfaces which can be inhibited by the addition of glucose. Recombinant banana lectin was able to induce systemic immunity by feeding, and Intramuscular administration without adjuvants led to the production of specific antibodies.

Conclusion:

Oral immunogenicity of banana lectin represents its inherent property which might be exploited for the induction of systemic immunity by the oral route.

GENERATION OF GENE-ENGINEERED CHIMERIC DNA MOLECULES FOR SPECIFIC THERAPY OF AUTOIMMUNE DISEASES

Vera Gesheva[1], Zsuzsanna Szekeres[2], Nikolina Mihaylova[1], Iliyana Dimitrova[1],

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Systemic lupus erythemathosus (SLE) is an autoimmune disease characterized by B cell hyperactivity. Delivering of a self-epitopes to the auto-reactive B cells involved in the pathological immune response has a negative effect on their activation. The specific elimination of dsDNA - recognizing B cells is a reasonable approach for effective therapy of SLE.

We have previously constructed a protein chimeric molecule by conjugation of DNA-mimotope peptides to a monoclonal anti-mouse CD32 (FcγRIIb) antibody. Using this protein-engineered molecule for therapy of lupus-prone MRL/lpr mice we suppressed selectively autoreactive B-lymphocytes by cross-linking B cell surface immunoglobulins with the inhibitory IgG FcγRIIb receptors (citat EJI). In the present study we have created a chimeric gene-engineered DNA molecule, encoding a single-chain variable fragment (scFv) from a monoclonal antibody against FcγRIIb, coupled to dsDNA-like peptide as a B-epitope. Such a DNA construct inserted in the expression vector pNut was used as a naked DNA vaccine in a mouse model of lupus. The DNA construct is able to be expressed in eukaryotic cells and to cross-link cell surface receptors on DNA-specific B cells, delivering an inhibitory intracellular signal.

Groups of lupus-prone MRL/lpr mice were injected intramuscularly with plasmid DNA encoding the chimeric molecule. The administration of the recombinant DNA molecule prevented the increase of IgG anti-DNA antibodies while in the control group they kept high levels. This result correlated with a low degree of proteinuria and preserved kidney histology in the chimera treated animals.

Keywords: Autoimmune therapy; Gene-engineered antibodies; Lupus;

IDENTIFICATION OF NCRNAS REGULATING DEVELOPMENT AND FUNCTION OF CD4$^+$ T CELLS

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Intensive study over the past few decades has considerably advanced our knowledge of the functional and phenotypic changes that occur throughout the T cell life cycle. There is evidence that epigenetic processes during development and differentiation establish cell fate. Nevertheless, knowledge of the underlying regulatory basis for T cell differentiation and function remains incomplete. Therefore, we collected RNA of naive and effector CD4$^+$ T cells as well as during T cell activation and differentiation in order to identify non-coding RNAs, which are of importance for differentiation and function of CD4$^+$ T cells, via genome-wide arrays.

Currently, we are evaluating these transcriptome data. We could already confirm the regulation of important key players of T cell activation within our data. Furthermore we found so far hypothetical proteins and unknown transcripts regulated following T cell stimulation. The induction of selected transcripts after activation of T cells could already be validated via real-time PCR.

IMMUNOREGULATORY ROLE OF GALECTIN-3

Ivan Jovanovic

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Galectin-3, a β galactoside–binding lectin plays an important role in processes relevant to the tumorgenesis such as malignant transformation, invasion and metastasis. We have investigated whether deletion of Galectin-3 in the host affects the metastasis of B16F1 malignant melanoma. Galectin-3-deficient (Gal-$3^{-/-}$) mice more resistant to metastatic malignant melanoma as evaluated by number and size of metastatic colonies in the lung. *In vitro* assays showed lower number of attached malignant cells in the tissue section derived from Gal-$3^{-/-}$ mice. Further, lack of Galectin-3 correlates with higher serum level of IFN-γ and IL-17 in tumor bearing hosts. Interestingly, Gal-$3^{-/-}$ mice have lower number of $Foxp3^{+}$ T cells after injection of B16F1 melanoma cells. Finally, we found that while $CD8^{+}$ T cell and adherent cell cytotoxicity were similar, there was greater cytotoxic activity of splenic NK cells of Gal-$3^{-/-}$ mice compared with "wild type" (Gal-$3^{+/+}$) mice. Despite the reduction in total number of $CD3\varepsilon^{-}NK1.1^{+}$, Gal-$3^{-/-}$ mice constitutively have a significantly higher percentage of effective cytotoxic $CD27^{high}CD11b^{high}$ NK cells as well as the percentage of immature $CD27^{low}CD11b^{high}$ NK cells. In contrast, $CD27^{low}CD11b^{high}$ less effective NK cells and NK cells bearing inhibitory KLRG1 receptor were more numerous in Gal-$3^{+/+}$ mice.

It appears that lack of Galectin-3 decrease capacity of melanoma cells to bind onto lung tissue and may prevent metastasis by enhancing NK-mediated anti-tumor response and tumor resistance in a malignant melanoma suggesting that Galectin-3 may be considered as therapeutic target.

A MONOCLONAL ANTIBODY DISTINGUISHES BETWEEN TWO IGM HEAVY CHAIN ISOTYPES IN ATLANTIC SALMON AND BROWN TROUT: PROTEIN CHARACTERIZATION, 3D MODELING AND EPITOPE MAPPING

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Atlantic salmon (Salmo salar) and brown trout (Salmo trutta) possess two distinct subpopulations of IgM which can be separated by anion exchange chromatography. Accordingly, there are two isotypic μ genes in these species, related to ancestral tetraploidy. In the present work it was verified by mass spectrometry that IgM of peak 1 (subpopulation 1) have heavy chains previously designated as μB type whereas IgM of peak 2 (subpopulation 2) have heavy chains of μA type. Two adjacent cysteine residues are present near the C-terminal part of μB, in contrast to one cysteine residue in μA. Salmon IgM of both peak 1 and peak 2 contain light chains of the two most common isotypes: IgL1 and IgL3. In contrast to salmon and brown trout, IgM of rainbow trout (Oncorhynchus mykiss) is eluted in a single peak when subjected to anion exchange chromatography. Surprisingly, a monoclonal antibody MAb4C10 against rainbow trout IgM, reacted with μA in salmon, whereas in brown trout it reacted with μB. It is plausible to assume that DNA has been exchanged between the paralogous A and B loci during evolution while maintaining the two sub-variants, with and without the extra cysteine. MAb4C10 was conjugated to magnetic beads and used to separate cells, demonstrating that μ transcripts residing from captured cells were primarily of A type in salmon and B type in brown trout. An analysis of amino acid substitutions in μA and μB of salmon and brown trout indicated that the third constant domain is essential for MAb4C10 binding.

This was supported by 3D modeling and was finally verified by studies of MAb4C10 reactivity with a series of recombinant μ3 constructs.

IMMUNIZATION WITH A DNA CHIMERIC MOLECULE ENCODING A HEMAGGLUTININ PEPTIDE AND A SCFV CD21-SPECIFIC ANTIBODY FRAGMENT INDUCES STRONG LONG-LASTING CTL RESPONSE TO INFLUENZA VIRUS

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Objectives and background:

DNA vaccination using naked DNA encoding viral antigens induces both humoral and cellular immune responses. There are several important advantages of the DNA vaccines over currently used protein vaccines, with the most significant being the fact that DNA vaccines induce a cytotoxic immune response as live attenuated viral vaccines do. We hypothesized that sequences encoding an epitope of influenza A virus hemagglutinin (IP) attached to sequences encoding a scFv antibody fragment against a co-stimulatory B cell surface receptor would result in the *in vivo* expression of a chimeric viral peptide with increased immunogenicity.

Methods:

We have inserted the DNA construct into pNut protein expression vector system. The constructs contained the Fab fragment of an antibody against mouse CR1/2 linked to IP peptide representing a conserved epitope of influenza A virus hemagglutinin and to the product of either the human oncogene products FOS or jun, having a natural tendency to bind to each another. This would result in the forming of more stable bivalent chimeric IP-carrying scFv molecules
Female Balb/C mice aged 8 to 10 week old were injected intramuscularly with a cocktail of 25μg of each construct per mouse. A complex immunization scheme was developed in which we apply one or combination of two types of vaccines.
Antibodies against the considered epitope in the sera of injected animals were measured with ELISA (eBioscience). The cell-mediated immune response induced in the animals was evaluated using a cytotoxic assay (Promega).

Results:

A recombinant DNA molecule was constructed, that encodes a T- and a B-cell epitope-containing influenza hemagglutinin peptide and a scFv antibody fragment that binds to mouse complement receptor. The construct was inserted into a protein expression system. An immunization with a plasmid containing the described construct induced a strong anti-influenza cytotoxic response lasting for more than 6 months. After prime-boosting with protein chimeric molecule we obtained anti-influenza cytotoxic and antibody response.

Conclusion:

Immunization of mice with pure DNA, encoding the antigen of interest attached to a scFv antibody fragment to mouse positive receptor, followed by prime-boosting has been successfully used to induce protective immunity against a model pathogen

AUTOREACTIVITY OF HUMAN NATURAL IGG AUTOANTIBODIES IS MASKED BY SERUM AND MUCOSAL IMMUNOGLOBULINS

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Objectives:

It is established that the immune system of healthy individuals is characterized by the presence of B-cells synthesizing IgG antibodies specific to a variety of self antigens. These natural IgG autoantibodies are present in the serum and in pooled human immunoglobulin preparations and recognize the antigens, which can be also targets for pathologic autoantibodies in autoimmune diseases. The induction of natural IgG autoantibody activity was observed after treatment with dissociating agents (for example - buffers with low or high pH value during elution from affinity column), but this induction of activity was not observed when non-denaturating methods of isolation were used.

Methods:

We have investigated the *in vitro* interactions between human serum from a healthy individual or pooled human IVIg and human liver antigens. Serum proteins from an individual serum and human colostrum were divided into fractions according to their molecular mass and pure IgA, IgM were isolated by two methods differing in the presence of low pH treatment of antibodies.

Results:

Purified serum or mucosal IgM, IgA as well as the fraction containing immunoglobulin F(ab')2 fragments inhibited in a dose-dependent manner the interaction between IgG obtained after affinity column isolation and also the interaction of pooled IVIg preparation and human liver antigens.

Conclusion:

The data show that the binding of auto-reactive IgG is inhibited in a dose-dependent manner by serum as well as by the mucosal IgA and IgM. Our study confirms previous findings regarding the role of IgM in blocking the self reactivity of IgG autoantibodies and supports the suggestion that a normal pooled human IgM preparation might possess a potent beneficial immunomodulatory activity in autoimmune patients as the control of auto-reactivity may be inefficient in some autoimmune diseases.

DIAGNOSIS AND SURVEY OF ALERGY CAUSED BY PARASITES IN ALBANIA

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Summary

Gastro-intestinal parasites infect more than 3 billion people worldwide. Most of them are widespread in developing heterogeneous areas composed of migrant population. According to studies of recent years, only 10% of these populations are carriers of 70% of intestinal helminthes.

One of the features associated with allergic diseases caused by parasites, in general is the increasing of concentration of IgE immunoglobulin in the serum of the patients. Because many immunoglobulins are closely associated with certain diseases, their use as diagnostic markers has been very successful. The frequency of cases in which the concentration of IgE immunoglobulin rises to various diseases is high, especially in those of allergic character. In the populations of industrialized countries in which the frequencies of parasitic infections are low, the action of IgE immunoglobulin faces high frequency reaction of the first type hypersensitivity. While in less developed countries dominated agrarian countries, parasitic infections are a major cause of increased concentrations of immunoglobulin IgE in serum.

Through the coproscopic method were analyzed as biological materials, the feces of 300 children from 1 to 14 years, for the presence of protozoa's eggs, helminthes larva, trophosoids, cists, etc. We have taken the photo of the positive cases. The analysis have been done in the Parasitological Laboratory of the Institute of Public Health, Tirana, through the method of concentration with floatation in sulphat zinc; the permanent color as Ziehln-Neelsen, Giemsa, Blu-metilen, etc.To determine the IgE are used the EIA kits. The level of the eosinophyle and IgE in the blood is performed in 76 individes who had been positive cases by helminthes.

Key words: parasites, protozoa, allergy, immunoglobulin, ELISA.

TARGET SILENCING OF DISEASE-ASSOCIATED B-LYMPHOCYTES BY CHIMERIC MOLECULES IN SCID MODEL OF PRISTANE-INDUCED AUTOIMMUNITY

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Objectives:

Systemic lupus erythemathosus (SLE) is a polygenic autoimmune disease characterized by B cell hyperactivity that leads to the generation of autoantibodies, formation of immune complexes, and clinical involvement of multiple organs. The current therapies of the disease are non-specific and more precise approaches targeting the disease-associated B lymphocytes, are urgently needed for clinical practice. Experimental therapy in humans is limited by technical and ethical restrictions. In contrast, studies in humanized mouse models can circumvent some of these limitations. Mouse models of autoimmunity are a tool to understand human pathology. Pristane-induced lupus is the only inducible model of autoimmunity associated with the clinical syndrome of SLE.

In the present study we test the effect of treatment with chimeric antibody, targeting disease-associated B lymphocytes only to pristane-induced autoreactive B cells. This study describes also a newly developed pristane-induced transferred SCID model of autoimmunity. This model allows the combination of pristane-induced autoimmune B or T cells from Balb/c mice with normal B or T cells from the same strain and modulation of the generated autoimmune response by a protein-engineered antibody.

Methods:

We constructed a chimeric antibody by coupling the dsDNA-mimicking peptides to a rat anti-mouse FcγRIIb monoclonal antibody to target disease-associated B lymphocytes only. The ability of the chimeric antibodies to suppress selectively the production of IgG anti-dsDNA antibodies were proven in *in vitro* and *in vivo* experiments.

Results:

Using the chimeric molecules in B (pristane) + T (pristane) transferred SCID model resulted in low level of IgG anti-DNA antibodies and of proteinuria during the treatment. In contrast, an increase in the urine protein concentration, anti-DNA antibodies and deposition of IgG-containing immune complexes in the glomeruli were observed in the PBS-injected controls during the same period. No pathologic kidney histology was detected in DNA-like chimera injected animals.

Conclusion:

In the present study we report a possible way to limit the interaction between autoimmune B and T cells, resulting in suppression of the lupus syndrome in pristane-induced cell-transferred SCID mice. The elimination of autoantigen-specific B cells could leave autoreactive T cells without potency of prolonged pathogenetic effects and restricts the progress of lupus disease in pristan-induced SCID model of autoimmunity.

A NOVEL CARBON MONOXIDE-RELEASING MOLECULE ALF421 ATTENUATES THE DEVELOPMENT OF AUTOIMMUNE DIABETES IN MICE INDUCED BY MULTIPLE LOW DOSES OF STREPTOZOTOCIN

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Carbon monoxide (CO), a by-product of heme catabolism by heme oxygenase, has recently been demonstrated to have potent anti-inflammatory and anti-apoptotic effects. ALF421 is a novel compound that is capable of modulating physiological functions *via* liberating of CO. However, its biological activity in autoimmune type 1 diabetes (T1D) has not been examined so far. Therefore, present study was conducted to investigate possible therapeutic value of ALF421 in the animal model of disease induced in susceptible C57BL/6 mice by multiple low doses of streptozotocin. Administration of ALF421 during diabetes induction, or even after the induction of the disease, suppressed the development of hyperglycemia. To further document cellular mechanism of beneficial effect of ALF421, we exposed mouse islets of Langerhans obtained from C57BL/6 mice, as well as rat (RIN) and mouse (MIN) insulinoma cells to proinflammatory cytokines (TNF-alpha+IL-1beta+IFN-gamma) in the presence or absence various doses of ALF 421 and analyzed their survival. These *in vitro* studies revealed that ALF421 in a dose-dependent way reduced cytokine-induced cell death in all three beta cell types tested, but did not affect basal cell viability. Although the molecular mechanisms involved in the drug action remain to be established, our results suggest that the observed beneficial effect of ALF421 in the disease process could be attributed at least partly to the interference of ALF421-released CO with cytokine-mediated pro-apoptotic stimuli within endocrine pancreas. Taken together, our results, showing for the first time cytoprotective role of ALF421 in experimental autoimmune diabetes, suggest that the drug may be useful in developing novel therapeutic strategy for prevention and early treatment of T1D.

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NEW THERAPEUTIC APPROACH TARGETING PATHOLOGICAL AUTOREACTIVE B LYMPHOCYTES AND PLASMA CELLS

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Objectives: A major disadvantage of all current therapeutic approaches used in autoimmune patients is the lack of specificity. We have previously presented at a bi-specific DNA-chimeric antibody that silences selectively autoreactive dsDNA-specific B lymphocytes in lupus mice. Recently, it has been shown that the proteasome inhibitor Bortezomib (which depletes the long-lived plasma cells) suppresses lupus activity in NZBxNZW F1 and in MRL/*lpr* mice. However, the prolonged depletion of all plasma cells is a highly undesirable event. We hypothesize that the periodical administration of a sub-toxic dose of a plasmacyte-depleting agent plus a chimeric antibody that specifically blocs the entry of new dsDNA-specific B cells into the pool of plasma cells, producing pathological autoantibodies would strongly suppress disease activity.
Methods: Groups of 7 weeks-old, disease-free female MRL/*lpr* mice were treated i.v. with sub-toxic doses of Bortezomib plus the bi-specific DNA-chimeric antibody. The effect of this combined treatment on anti-dsDNA antibody- and proteinuria levels and on lymphadenopathy was compared to that of Bortezomib, of the DNA-chimeric antibody and of Cyclophosphamide administered as mono-therapies. Selected spleen B cell subpopulations - plasma cells (CD19-CD138+), germinal (CD19+PNA+), follicular ($CD19+CD23^{hi}CD21^{low}$), marginal ($CD19+CD23^{low}CD21^{hi}$), and mature (CD19+IgM+IgD+) B lymphocytes and plasmablasts (CD19+CD138+) were studied by flow cytometer.
Results: The simultaneous administration of Bortezomib plus the DNA-chimeric antibody resulted in decreased levels of anti-dsDNA IgG antibodies, of proteinuria, in prevention of the appearance of the typical inflammatory skin lesions and in reduced sizes of the spleens and lymph nodes of the treated animals. During the climax of the disease (at 18 weeks of age) the histological analysis showed no immune complex deposition in the glomeruli of the animals from this group. At this age the simultaneous treatment with the proteasome inhibitor plus the DNA-chimeric antibody significantly reduced all analyzed subpopulations with the exception of the germinal B-lymphocytes and of plasmablasts.
Conclusion: Our results strongly suggest that the effect of simultaneous administration of an agent targeting the disease-associated dsDNA-specific B lymphocytes and of a non-toxic dose of a plasma cell-depleting drug, is superior to that of presently used treatment approaches.

NITRIC OXIDE (NO) INHIBITS CXCL12 GENE EXPRESSION IN SPINAL CORD MICRO BLOOD VESSELS AND ASTROCYTES OF A RAT

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Nitric oxide is an important effector and/or regulatory molecule in immune reactions, mainly synthesized by inducible nitric oxide synthase (iNOS). CXCL12 is a chemokine that shows anti-inflammatory mode of action in experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis, but the mechanisms regulating gene expression and production of this molecule are poorly understood

.

The aim of the work was to test effects of NO on CXCL12 gene expression in micro blood vessels of a spinal cord and astrocytes, as these are major sources of this molecule in central nervous system (CNS). We were estimating gene expression rates of iNOS and CXCL12 in spinal cord homogenates of rats that developed EAE. Astrocytes, C6 cell line (rat astrogliom) and micro blood vessels were stimulated with pro-inflammatory cytokines and sodium-nitroprusside (NO donor), and gene expression was studied using RT- ''real time'' PCR. The level of NO that was produced in cell cultures was measured by ''Griess reaction''.
We have determined negative correlation between iNOS and CXCL12 gene expressions in spinal cord homogenates. In addition, it has been shown that NO inhibits CXCL12 gene expression in micro blood vessels, astrocytes and C6 cell line in vitro.

Nitric oxide overproduction during neuroinflammation could contribute to inhibition of CXCL12 expression in CNS and reduce anti-inflammatory role of this chemokine.

TRICHINELLA SPIRALIS MUSCLE LARVAE ANTIGENS REACTIVE WITH HUMAN AUTOANTIBODIES

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Infections have been implicated in the onset and/or promotion of autoimmunity. On the other hand, there is growing body of evidence that some infections modulate immune response in a way to ameliorate or even protect from autoimmune disease development. Helminths, like *Trichinella spiralis (T. spiralis)*, are regarded as master manipulators of the host immune system. Besides provoking strong and specific immune response, infection with this parasite is capable to modulate response to irrelevant antigens and to tame several autoimmune disorders in rodent models, as has been shown by us and other authors. We have also noticed that excretory-secretory components of the muscle larvae (ES L1) are involved in the creation of immune response dominated by regulatory network that controls the development of inflammatory diseases. During co-evolution, *T. spiralis*, like other helminths, developed strategies to survive in host organism by induction of tolerogenic status, most probably through components that express epitopes with stereochemical configuration that resemble those on host antigens, either expressed or hidden. To reply on the question which of *T. spiralis*-derived products could be responsible for provoking 'tolerogenic' responses e.g. are there evolutionary conserved antigens among parasite and host that may be implicated in the phenomenon, we examined reactivity of *T. spiralis* muscle larvae antigens with 6 different types of autoantibodies. The existence of autoantibodies that cross-react with *T. spiralis* antigens was recognized by immunohistological screening in 24 (43.6 %) out of 55 analyzed human sera. The same group of sera expressed reactivity with total number of 24 protein components of ES L1 antigens in the Western blot, with very broad spectrum of molecular masses (14 to 130 kDa). Human autoantibodies bound predominantly antigens that belong to TSL1 group, to the greatest extent 53 kDa component of ES L1 (14 out of 24 sera, 58.3%) and, in gradation decrease, 3 other antigens inside the group (43 kDa - 41.7%; 49 kDa and 45 kDa - 25% each). These ES L1 protein components could be good candidates for further studies on mechanisms involved in immunomodulation driven by *T. spiralis.*

MULTIDRUG RESISTANCE IN INFLAMMATORY BOWEL DISEASE. LOCALIZATION AND EXPRESSION PROFILES OF ABC TRANSPORTERS PGP AND MRP1 IN GASTROINTESTINAL TRACT

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Background and aims:

Inflammatory bowel disease (IBD) refers to inflamation of small or large intestine and can be manifested in two distinct forms: Ulcerative colitis (UC) and Crohn's disease (CD). Whereas CD is a discontinuous transmural chronic inflammatory disorder that can affect any area of the gastrointestinal tract, the UC is confined to the top layers of the colon and rectum. The exact pathophisiology is unknown but it includes environmental and immunological effects on genetic succeptible individual. Treatment includes antibiotics, anti infflamatory drugs, corticosteroids, immune system supressors and monoclonal antibodyes like Infliximab. Although treatment can lead to remission of the disease a multidrug resistance (MDR) can occur. There are many mechanism that can lead to MDR among which is overexpression of ABC transporters. Because amplified efflux of orally administered drugs reduces it's bioavailability and glucocorticoids are known supstrates of MDR1 (P-glycoprotein, P-gp) we set to assess the expression profiles of P-gp and MRP1 at 6 different locations (terminal ileum, ascending, transverse and descending colon, sigma and rectum) of the gastrointestinal tract (GIT) and in further examinations of same patients see if the treatment leads to increased expression profile of each transporter which could explain the MDR occurence.

Materials and methods:

Total RNA was extracted from intestinal biopsies of 15 Crohn patients and 7 healthy controls upon their informed consent in writing, according to the Helsinki Declaration and appproved by the Hospital Ethics Committees. All patients were newly diagnosed without any type of cytoredu-ctive treatment prior the study. Gene expression was investigated by quantitative real-time PCR (TaqMan).

Results:

Both transporters showed higher expression in controls compared to CD, especially at terminal ileum for mdr1 gene ($p<0.05$). The expression of mdr1 gene was highest at terminal ileum and than droped and remained aproximatelly the same. MRP1 expression was constant at all 6 locations. In all segments of the colon the expression level of mdr1 was higher than mrp1 but reached statistical significance only at terminal ileum ($p>0.05$).

Conclusions:

Lower expression of both transporters in CD can be the result of inflamation proces. These results do not exclude the posibility that increased expression of transporters contribute to MDR because all of the CD patients were newly diagnosed. It will be interessant to follow up those patients with lower pre-treatment values to check the hypothesis.

TNF ALPHA - 308 GENE POLYMORPHISM IN SERBIAN PATIENTS WITH PSORIASIS VULGARIS

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Objectives:

Tumor necrosis factor alpha (TNF) has been considered the prototypic cytopathogenic cytokine in many autoimmune diseases including Psoriasis vulgaris (PV). It has been reported that the TNF –308*G/A polymorphism (rs1800629) influences levels of TNF production, and that the rare allele, TNFA, is associated with high TNF production. We investigated the TNF –308*G/A polymorphism in 55 unrelated Serbian patients with PV and 132 ethnically matched, healthy individuals.

Methods:

Blood samples were obtained from patients and healthy controls. DNA was isolated by genomic DNA purification kit (Fermentas). We used sequence- specific primer - PCR approach to determine the TNF –308*G/A polymorphism.

Results:

PV patiens had higher frequency of the TNFA allele in comparison with controls (19.1% vs. 11.8 %) but the difference did not reach the level of statistical significance (p = 0.06). Genotype distribution in patients and controls was similar (p=0.13). TNF –308*G/G genotype was present in 36 patients (65.5%) and 102 controls (77.3%), TNF –308*G/A in 17 (30.9%) patients and 29 (22.0 %) controls) and TNF –308*A/A homozygous were 2 patients (3.6%) and 1 control individual (0.7%).

Conclusion:

In so far analyzed Serbian PV patients, there was no statistically significant difference in either allele or genotype frequency for TNF –308*G/A polymorphism as compared to healthy controls. However, there is a tendency of higher frequency of the TNFA allele in PV patients in this ongoing study, yet it is to be assessed in a larger cohort.
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THE ROLE OF ETHANOL-INDUCED CELL STRESS IN REGULATION OF MICA/B PROTEIN EXPRESSION

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Expression of MICA/B ligands of the NKG2D immunoreceptor has been proposed to play an important role in the detection and suppression of tumors. MIC proteins (MHC class I homolog) are stress-inducible and expressed on the surface of tumor, infected or stressed cells. The result of the interaction of MICA/B with NKG2D receptor is the increase of cytotoxic activity of NK- and T-cells and the subsequent killing of affected cells. On the contrary, soluble MICA/B (sMICA/B) can depress NK cell functions particularly because of decrease of NKG2D surface expression.

In this work the expression of MICA/B was analyzed in a model of ethanol-induced cell stress using K562, Jurkat and THP-1 tumor cell lines as well as human peripheral mononuclear cells. Surface MICA/B expression was analyzed by flow cytometry and confocal microscopy. Intracellular pool of the proteins was registered by confocal microscopy. Expression of mica/b genes was detected by RT-PCR. In contrast to Jurkat cells, the significant level of spontaneous surface MICA/B was registered in K562 and THP-1 cells. Surface expression of MICA/B increased under the influence of ethanol-induced cellular stress (0.5-1%) and hyperthermia. Then surface MICA/B expression decreased, with ethanol dose increasing up to 2% and above. The process was corresponded to cell death elevation.

We did not found spontaneous surface expression of MICA/B in lymphocytes, but discovered it in CD14-positive cells, increased under the influence of ethanol-induced cellular stress (0.12-1%). Those cells, which have no spontaneous MICA/B surface, have a considerable level of mRNA mic. With confocal microscopy, it was shown that under the influence of ethanol-induced cellular stress the inner pool of MICA/B decreased and the surface pool increased in comparison with the control cells. We can assume that, under the influence of alcohol, MIC moved from the cytoplasm to the cell surface.

Thus, we have shown ethanol-induced increase of MIC expression in some tumor cells and normal monocytes, which may have a modulating effect on the immune system.

GALECTIN-3 DEFICIENCY PREVENTS CONCANAVALIN A- INDUCED HEPATITIS IN MICE

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Background and aims:

We used Concanavalin A induced liver injury to study the role of Galectin 3 in the induction of inflammatory pathology and hepatocellular damage.

Methods:

We tested susceptibility to Concanavalin A induced hepatitis in Galectin 3 deficient and analyzed the effects of pre-treatment with selective inhibitor of Galectin 3(Gal3 INH) in wild type C57BL/6 mice as evaluated by liver enzyme test, quantitative histology, mononuclear cell infiltration, cytokine production, intracellular staining of immune cells and percentage of apoptotic mononuclear cells (MNCs) in the liver.

Results:

Galectin 3 deficient mice are less sensitive to Con A induced hepatitis and had significantly lower number of MNCs in the liver, CD3+, CD4+ and CD8+ T cells, CD19+ B cells, NK1.1+ NK and NK1.1+CD3+ NKT cells, CD11c+ DCs and CD11c+CD80+CD86+ activated DCs. However, there was no significant difference in total number of CD4+CD25+Foxp3+ T regulatory cells and F4/80+ macrophages between WT and Gal-3-/- mice. The level of TNF alpha, IFN gamma, IL-17 and IL-4in the sera and number of TNF alpha, IFN gamma, IL-17 and IL-4 producing CD4+ cells, IL-12 producing CD11c+ DCs, were lower in Galectin 3 deficient mice. In contrast, number of IL-10 producing F4/80+ macrophages was significantly higher in liver of Gal-3-/- mice. Further, apoptosis of infiltrating cells contributes to the lower number of MNCs in liver of Gal-3 -/- mice because significantly higher percentage of late apoptotic Annexin V+ PI+ liver infiltrating MNCs and splenocytes were seen in Gal-3-/- mice compared with WT mice. Pre-treatment of Wild type C57BL/6 mice with Gal3 INH led to attenuation of the liver injury and milder infiltration of IFN gamma, IL-17 and IL-4 producing CD4+ T cells, increase in total number of IL-10 producing CD4+ T cells and F4/80+ CD206+ alternatively activated (M2 polarized) macrophages and prevented apoptosis of liver infiltrating MNCs

Conclusions:

We concluded that Gal-3 plays an important pro-inflammatory role in Con A induced hepatitis by promoting activation of T lymphocytes, NKT cells and maturation of DCs, secretion of pro-inflammatory cytokines and down-regulating M2 macrophage polarization and apoptosis of MNCs in the liver.

Key words:

Galectin 3, Galectin 3 INH, C57BL/6 mice, Gal3 deficient mice, Concanavalin A, hepatitis

A MOUSE MODEL OF INTRANASAL S. PNEUMONIAE, SEROTYPE 6B, INFECTION

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Introduction

Animal models of infectious disease are widely used in biomedical research but no cookbook model can be applied due to the myriad array of influencing factors for each scenario. The aim of this study was to realize an intranasal model of S. pneumoniae 6B infection in hyaluronidase primed C57BL6 mice.

Materials and Methods

Adult female C57BL6 mice were infected intranasally with 1, 2 or 3 repeated doses of S. pneumoniae, serotype 6B, after a hyaluronidase pretreatment under mild anesthesia. Breathing parameters, bacterial presence and pulmonary gross and histopathological changes in lungs were assessed on day 1, 2, 4, 6 and 10 post-infection. Body weight and classic clinical signs of pneumonia where monitored daily during the experiment.

Results

A decrease in mean tidal volume was observed till up to 4 days after infection with one dose of bacteria while two doses decreased mean tidal volume till up to 4 days.

S. pneumoniae positive samples were obtained from one mouse of each group at 24h post infection from both blood and lungs, confirming the successful infection. Blood and lung cultures were positive for S. pneumonia 6B at each time point in at least one randomly picked mouse from each group.

Pathological changes in the lungs did not include gross modifications of organs but histopathologic findings showed evidence of inflammation in correspondence with bacterial inoculums and the moment post infection at which mice were sacrificed.

Body weight drop and specific clinical signs were not detected in infection or hyaluronidase control groups.

Conclusions

Under our protocol the results show that S. pneumoniae 6B causes subclinical pneumonia in C57BL6 mice that affects breathing parameters due to infectious inflammatory process within the lung.

Whole body pletysmography can be a useful in-vivo method for assessing severe respiratory disease but it does not have the sensibility to pick up on minute changes in a subclinical pulmonary condition.

This study was supported by the PN 09220201 grant funded by the Ministry of Research and Education.

HEREDITARY ANGIOEDEMA, CLINICAL CHARATERISTICS AND DISTRIBUTION IN POPULATION OF MONTENEGRO /PROJECT/

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Intro:

Hereditary angioedema (HAE), a rare but life-threatening#condition, manifests as acute attacks of facial, laryngeal, genital, or peripheral swelling or abdominal pain secondary to intra-abdominal edema. Resulting from mutations affecting C1 esterase inhibitor (C1-INH), inhibitor of the first complement system component, attacks are not histamine-mediated and do not respond to antihistamines or corticosteroids. Inheritance is autosomal-dominant, it was initially described by Osler in 1888.

Aim:

Objectives of our study are:

1) Defining laboratory and clinical charateristics of patients with diagnosed HAE according to the current criteria
2) Screening of family members and relatives who are under risk, determinig gender and age distribution, inheritance rate, defining laboratory and clinical charateristics of newly discovered patients
3) Forming a official register for HAE in state of Montenegro
4) Education of patients about prevention, trigger factors (dental and surgical interventions etc), information about profilactic therapy, decreasing acute attack complications

Method and material:

Our study will include all diagnosed patients on the teritory of Montenegro, their family members and relatives.

Patients and their relatives will be evaluated by: questionnaire,family tree schemes, clinical examinations, laboratory tests. These tests include nephelometry (C3, C4), radial immunodiffusion (C1 inhibitor, C1q), ELISA (anti C1q), and indirect immunofluorescence microscopy for antinuclear antibodies (ANA). Tests will be performed in the Laboratory for immunology in the Clinical center of Montenegro. Data will be analized by descriptive statistics and parametric and nonparametric tests.

Discussion and results:

Obtained results will be compared with the literature data, so it will determine accurate incidence of HAE in Montenegro, while the expected one is 1/50000-150000. Evaluation of attacks severity and number of endangered patients will stress out need for registering necessary medications (human or recombinant C1 inh, ecallantide, icatibant). It will also provide calculation for consumption and planning.