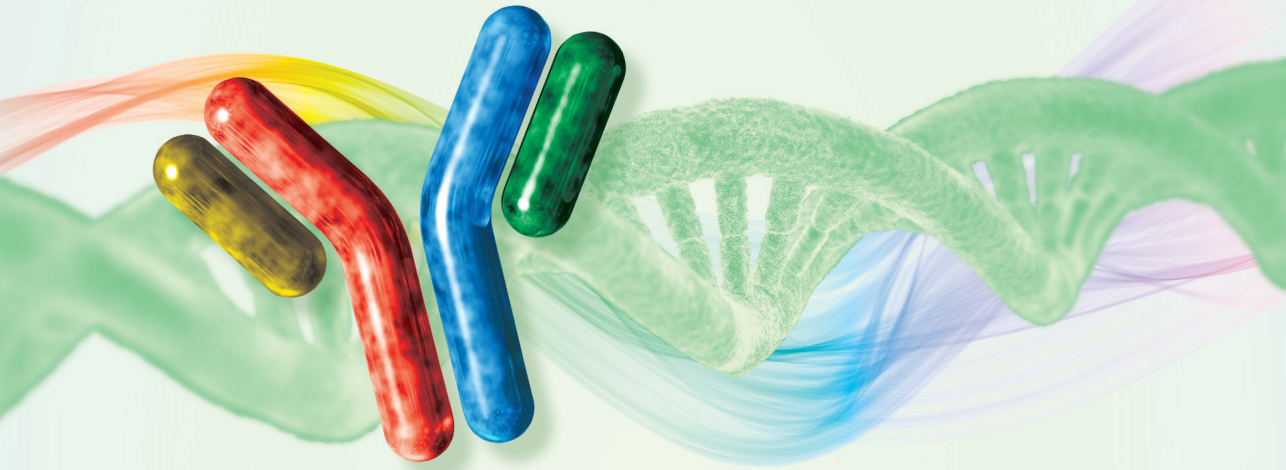
XVII Congress of the Polish Society of Experimental and Clinical Immunology

Book of Abstracts



Medical University of Białystok, Poland

May 27-29th, 2021

ORGANIZER:

Main Board of the Polish Society of Experimental and Clinical Immunology

Bialystok Chapter of the Polish Society of Experimental and Clinical Immunology

HONORARY PATRONAGE:

Rector of the Medical University of Białystok

Mayor of Bialystok

ORGANIZATIONAL PARTNER:

mediteka

www.ptidik2021-bialystok.pl

INFARMA
Związek Pracodawców
Innowacyjnych Firm Farmaceutycznych

Certyfikat nr 3858

Wydarzenie:

XVII Congress of the Polish Society of Experimental and Clinical Immunology

Odbywające się w: wydarzenie online
W dniach: 27 - 29 maja, 2021

spełnia standardy etyczne wynikające z Kodeksu Przejrzystości
oraz Kodeksu Dobrych Praktyk Przemysłu Farmaceutycznego

Dyrektor Generalny INFARMA

Bogna Cichowska-Duma

WYDARZENIE INFARMA 3858 CERTYFIKOWANE

Związek Pracodawców INFARMA reprezentuje 27 działających w Polsce wiodących firm sektora farmaceutycznego, prowadzących działalność badawczo-rozwojową i produkujących leki innowacyjne. INFARMA jest członkiem międzynarodowych organizacji zrzeszających innowacyjną branżę farmaceutyczną (EFPIA), a także Pracodawców RP oraz Krajowej Izby Gospodarczej.

Dear Friends and Colleagues from Polish and International Immunological Community,

On behalf of the Board of the Polish Society of Experimental and Clinical Immunology and the Białystok Chapter of the Society, we would like to cordially invite you to the ***17th Congress of the Polish Society of Experimental and Clinical Immunology****. The congress will be held online from the 27th to 29th of May 2021. The venue of the Congress will be the Medical University of Bialystok.*

Together with acclaimed experts from Poland and abroad, and the attendants of the Congress, we will build up an interesting scientific programme including the latest developments in basic and clinical immunology, tackling such issues as primary and secondary immunodeficiencies, congenital immunodeficiency, immunoregulation, autoimmune and autoinflammatory diseases, cancer immunology and immunotherapy, immunology in personalized medicine, reproductive immunology, immunology of aging, immunotoxicology and immunology of COVID-19. The programme will also include sessions on allergology and methods of allergen therapy.

We are looking forward to your active participation in the Congress and interesting propositions for lectures and poster presentations. We hope that the Congress will be a successful and productive event of high scientific value, and will have a positive impact on the progress in immunology.

President of Polish Society of Experimental and Clinical Immunology:

Prof. Maciej Kurpisz MD, PhD
Institute of Human Genetics, Polish Academy of Sciences, Poznań

Chairman of the Scientific Committee:

Prof. Jacek M. Witkowski MD, PhD
Medical University of Gdańsk

Chairpersons of the Organizing Committee:

Prof. Ewa Jabłońska MD, PhD
Medical University of Białystok

Prof. Marcin Moniuszko MD, PhD
Medical University of Białystok

SPONSORS & EXHIBITORS

MAIN SPONSORS

BERLIN-CHEMIE MENARINI

STEMCELL™ TECHNOLOGIES

Polski Bank Komórek Macierzystych FamiCord Group

Takeda

SPONSORS

AstraZeneca

gsk

KEDRION BIOPHARMA

EXHIBITORS

allergopharma-nexter

CSL Behring

frontiers in Aging

sobi rare strength

PARTNERS

cedus
Centrum Diagnostyki Obrazowej
Szkolenia dla lekarzy

medipage
wydawnictwo medyczne

Scientific Committee:

Chairman: Jacek Witkowski, Gdansk, Poland
Jarosław Baran, Cracow, Poland
Ewa Bernatowska, Warsaw, Poland
Przemysław Błyszczuk, Cracow, Poland
Katarzyna Bogunia-Kubik, Wroclaw, Poland
Ewa Bryl, Gdansk, Poland
Krzysztof Bryniarski, Cracow, Poland
Joanna Cichy, Cracow, Poland
Krystyna Dąbrowska, Wroclaw, Poland
Urszula Demkow, Warsaw, Poland
Joanna Domagała-Kulawik, Warsaw, Poland
Andrzej Górski, Wroclaw, Poland
Ewelina Grywalska, Lublin, Poland
Ewa Jabłońska, Bialystok, Poland
Karina Jahnz-Różyk, Warsaw, Poland
Jolanta Jura, Cracow, Poland
Marek Jutel, Wroclaw
Lidia Karabon, Wroclaw, Poland
Sylwia Kołtan, Bydgoszcz, Poland
Zdzisława Kondera-Anasz, Poznan, Poland
Magdalena Klink, Lodz, Poland
Krzysztof Kowal, Bialystok, Poland
Marek Kulus, Warsaw, Poland
Maciej Kurpisz, Poznan, Poland
Andrzej Lange, Wroclaw, Poland
Witold Lasek, Warsaw, Poland
Andrzej Mackiewicz, Poznan, Poland
Jacek Malejczyk, Warsaw, Poland
Janusz Marcinkiewicz, Cracow, Poland
Bożena Mikołuć, Bialystok, Poland
Marcin Moniuszko, Bialystok, Poland
Krzysztof Mucha, Warsaw, Poland
Paulina Niedźwiedzka-Rystwej, Szczecin, Poland
Małgorzata Pac, Warsaw, Poland
Marcin Pasiarski, Kielce, Poland
Anna Pituch-Noworolska, Cracow, Poland
Zygmunt Pojda, Warsaw, Poland
Mariusz Ratajczak, Warsaw, Poland
Wioletta Ratajczak-Wrona, Bialystok, Poland
Jacek Roliński, Lublin, Poland
Maciej Siedlar, Cracow, Poland
Jan Sikora, Poznan, Poland
Andrzej K. Siwicki, Olsztyn, Poland
Jacek Szepietowski, Wroclaw, Poland
Magdalena Trzeciak, Gdansk, Poland
Beata Tokarz-Deptuła, Szczecin, Poland
Piotr Trzonkowski, Gdansk, Poland
Beata Wolska-Kuśnierz, Warsaw, Poland
Radosław Zagożdżon, Warsaw, Poland
Joanna Zajkowska, Bialystok, Poland
Krzysztof Zeman, Lodz, Poland
Jan Żeromski, Poznan, Poland

Members of the Organizing Committee:

Chairperson: Ewa Jabłońska
Chairperson: Marcin Moniuszko
Andrzej Eliaszewicz
Marzena Garley
Kamil Grubczak
Agnieszka Iwaniuk
Kinga Henryka Nowacka
Karolina Nowak
Wioletta Ratajczak-Wrona
Aleksandra Starosz
Marlena Tynecka

XVII[TH] CONGRESS OF THE POLISH SOCIETY FOR FUNDAMENTAL AND CLINICAL IMMUNOLOGY

PROGRAM

<table>
<tr><th colspan="3">DAY 1: Thursday, May 27, 2021</th></tr>
<tr><td colspan="3">08:30–10:00 Opening ceremony</td></tr>
<tr><td colspan="3">Plenary lecture</td></tr>
<tr><td colspan="3">The role of MHC and associated proteins in reproduction
Nelson Fernandez, Essex, UK</td></tr>
<tr><td colspan="3">10:00–11:30 Parallel Session</td></tr>
<tr><td rowspan="2">Basic Immunology</td><td colspan="2">Clinical Immunology and Immunotherapy</td></tr>
<tr><td>Part I</td><td>Part II</td></tr>
<tr><td>Innate immunity in protection of barrier sites and internal organs
Chairs:
Janusz Marcinkiewicz, Cracow, Poland
Joanna Cichy, Cracow, Poland</td><td>The expanding field of secondary immune deficiencies in adults: causes, diagnosis, and management
Chairs:
Jacek Roliński, Lublin, Poland
Marcin Pasiarski, Kielce, Poland</td><td>Immunology in personalized medicine
Chair:
Ewa Bryl, Gdansk, Poland</td></tr>
<tr><td colspan="3">10:00–11:30 Parallel Session</td></tr>
<tr><td>Immunomodulation and Immunotoxicology
Chairs:
Andrzej Siwicki, Olsztyn, Poland
Ewa Jabłońska, Bialystok, Poland</td><td>Progress in diagnostics and treatment of primary immunodeficiencies
Chairs:
Ewa Bernatowska, Warsaw, Poland
Małgorzata Pac, Warsaw, Poland</td><td>Rheumatology
Chairs:
Ewa Bryl, Gdansk, Poland
Maciej Siedlar, Cracow, Poland</td></tr>
<tr><td colspan="3">14:00–15:00 Plenary lecture</td></tr>
<tr><td colspan="3">CAR-T cell approaches to AML treatment
Yupo Ma, NY, USA</td></tr>
<tr><td colspan="3">15:00–16:30 Parallel Session</td></tr>
<tr><td>Current advancements in basic immunology
Chair:
Krzysztof Bryniarski, Cracow, Poland</td><td>Tumor immunology and immunotherapy
Chairs:
Andrzej Mackiewicz, Poznan, Poland
Joanna Domagala-Kulawik, Warsaw, Poland
Witold Lasek, Warsaw, Poland</td><td>Modern diagnostics of immune-related diseases
Chairs:
Urszula Demkow, Warsaw, Poland
Jarosław Baran, Cracow, Poland</td></tr>
<tr><td colspan="3">16:45–17:45 Plenary lecture</td></tr>
<tr><td colspan="3">Tumor-derived exosomes and their role in cancer progression and response to immune therapy
Theresa L. Whiteside, Pittsburgh, USA</td></tr>
</table>

17:45–19:15 Parallel Session		
New aspects of innate immunity in cancer Chairs: **Ewa Jabłońska**, Białystok, Poland **Magdalena Klink**, Łódź, Poland	**Autoimmunity and Autoinflammation** Chairs: **Karina Jahnz-Różyk**, Warsaw, Poland **Beata Wolska-Kuśnierz**, Warsaw, Poland	**Immunology of infection** Chairs: **Beata Tokarz-Deptuła**, Szczecin, Poland **Andrzej K. Siwicki**, Olsztyn, Poland

DAY 2: Friday, May 28, 2021

9:00–10:00 Plenary lecture

Targeting an interface between innate and adaptive immunity in protecting from target organ damage in hypertension and atherosclerosis
Tomasz Guzik, Glasgow, UK

10:00–11:30 Parallel Session		
Basic Immunology	**Clinical Immunology and Immunotherapy**	
	Part I	Part II
Immunity, aging and aging-related diseases Chair: **Jacek M. Witkowski**, Gdansk, Poland	**Vaccinations in Immunocompromised** Chairs: **Ewa Bernatowska**, Warsaw, Poland **Bożena Mikołuć**, Bialystok, Poland	**Immunology of reproduction** Chairs: **Maciej Kurpisz**, Poznan, Poland **Jacek Malejczyk**, Warsaw, Poland

11:45–13:20 Parallel Session		
Bacteriophages and Immunity Chairs: **Andrzej Górski**, Wroclaw, Poland **Krystyna Dąbrowska**, Wroclaw, Poland	**Allergology** Chairs: **Marek Kulus**, Warsaw, Poland **Marcin Moniuszko**, Bialystok, Poland	**Adoptive cell therapy** Chairs: **Radosław Zagożdżon**, Warsaw, Poland **Krzysztof Mucha**, Warsaw, Poland

13:20–13:40 TBA – Sponsored lecture

Takeda

13:40–14:00 TBA – Sponsored lecture

Berlin-Chemie

14:00–15:00 Special Session

Polish Immunology: from past to the future
Chairs:
Jan Żeromski, Poznań, Poland; **Jacek M. Witkowski**, Gdańsk, Poland

15:00–17:00 Parallel Session		
Basic Immunology	**Clinical Immunology and Immunotherapy**	
	Part I	Part II
Immune tolerance – from bench to bedside Chair: **Piotr Trzonkowski**, Gdansk, Poland	**Allergen immunotherapy and biological treatment** Chairs: **Karina Jahnz-Różyk**, Warsaw, Poland **Marek Jutel**, Wroclaw, Poland	**Advances and challenges of immunogenetics towards personalized therapy** Chairs: **Katarzyna Bogunia-Kubik**, Wroclaw, Poland **Lidia Karabon**, Wroclaw, Poland

17:15–18:15 Poster session 1

18:15–19:15 Poster session 2

DAY 3: Saturday, May 29, 2021

9:00–10:00 Plenary lecture

Progress in the production of therapeutic haematopoietic cells from pluripotent stem cells
Lesley Forrester, Edinburgh, UK

10:00–11:30 Parallel Session

Basic Immunology	Clinical Immunology and Immunotherapy	
	Part I	Part II
Immunity and stem cells Chairs: **Mariusz Ratajczak**, Warsaw, Poland **Marcin Moniuszko**, Bialystok, Poland	**Immunopathology** Chairs: **Zdzisława Kondera-Anasz** **Jan Sikora**, Poznan, Poland	**Unobvious use of immunoglobulins** Chairs: **Anna Pituch-Noworolska**, Cracow, Poland **Małgorzata Pac**, Warsaw, Poland

12:00–14:00 Parallel Session

Veterinary immunology and comparative immunology Chairs: **Andrzej Siwicki**, Olsztyn Poland **Paulina Niedzwiedzka-Rystwej**, Szczecin, Poland	**Immunodermatology** Chairs: **Jacek Szepietowski**, Wrocław, Poland **Jolanta Jura**, Cracow, Poland	**Immunology of COVID-19** Chairs: **Joanna Zajkowska**, Bialystok, Poland **Janusz Marcinkiewicz**, Cracow, Poland

14:00–14:30 Closing remarks

Table of Contents

Plenary lectures

The role of MHC and associated proteins in reproduction

Nelson Fernández, Isra Modawi and Asma Jabeen

School of Life Sciences, University of Essex, Colchester, CO4 3SQ, England

EThe MHC plays a key role in mammalian reproduction at, both population levels, acting as a selection pressure and at the feto-maternal interface, during pregnancy development. However, in humans, the MHC is selectively expressed during pregnancy, some loci are switched off and others are expressed. For example, the combined haplotype and individual function of the loci encoding HLA-C, HLA-G, HLA-E and HLA-F are fully expressed but their role remains elusive. The encoded molecules are confined to extra villous trophoblasts throughout gestation, the cells that invade the *decidual stroma* (interstitial invasion) and the maternal spiral arteries, that serve to open the uterine spiral arteries suggesting a possible role in angiogenesis. In early ontogenic studies we showed that in mice MHC Class I genes are transcribed at pre-implantation level (1). It has been proposed that in humans, HLA Class I subsets protect the fetus by promoting trophoblast-uterine effector cell interactions; the precise mechanisms are unknown. Phenotypic studies revealed that trophoblasts lack expression of the highly polymorphic loci HLA-A and HLA-B, both loci encode molecules that act as T-cell ligands, presumably allowing the foetus to escape allograft rejection and allo-recognition by the maternal immune system, that otherwise would stimulate maternal anti-foetal CD8+ cytotoxic T lymphocytes causing a local adverse inflammatory process. An alternative conjecture is that regulation of trophoblast function operates principally via uterine natural killer (uNK) cells rather than T-cells. HLA-C, together with HLA-G, HLA-E and HLA-F, play a functional role in uNK cell recognition and inhibition. This would allow optimal protection against immune responses to paternal HLA antigens during pregnancy. We have shown that human trophoblast choriocarcinoma cells co-express HLA-G and HLA-E and are simultaneously up-regulated (2,3). Using advanced bioimaging techniques developed by our group at Essex, we have observed that these molecules colocalize with each other on the plasma membrane and have the potential to form preferential physical associations. We term these associations as *heterotypic associations*, since they are between different allelic products, e.g. HLA-G colocalizes with HLA-E. These findings led us to propose a model in which HLA Class I molecules are able to form clusters and heterotypic associations (4) and are subject to immunomodulation by cytokines, progesterone and pre-implantation soluble factors (5,6). Selective HLA expression, coupled with physical association of pairs of these antigens, might indicate that placental trophoblasts function as biosensors that contribute to foetalmaternal tolerance. One challenge is to evaluate if those colocalized heterotypic HLA Class I proteins on trophoblast cells amplify the inhibitory signal delivered to NK cells, thus blocking their effector activity allowing pregnancy to develop.

(1) Joanne C Cooper, Gillian B Dealtry, Petra C Arck, Sandra M Blois and Nelson Fernández. (2007) Identification of an impaired breeding phenotype in mice with a genetic deletion of 2-microglobulin and diminished MHC Class I expression: a role in MHC dependent reproductive fitness. *Biology of Reproduction* 77: 274-279.

(2) Shaikly, V.R., Morrison, I.E.G., Taranissi, M., Noble, C.V., Withey, A.D., Cherry, R.J., Blois, S.M. and Fernández, N. (2008). Analysis of HLA-G in maternal plasma, follicular fluid, and preimplantation embryos reveal an asymmetric pattern of expression *J. Immunol.* 180:4330-4337.

(3) Shaikly, V.R., Shakhawat, A., Withey, A., Morrison, I., Taranissi, M., Dealtry, G.B., Jabeen, A., Cherry, R. and Fernández, N. (2010). Cell bio-imaging reveals co-expression of HLA-G and HLA-E in human preimplantation embryos. *Reprod. Biomed. Online*, 20: 223-233.

(4) Obara, B., Jabeen, A., Fernández, N. and Laissue, P.P. (2013). A novel method for quantified, superresolved, three-dimensional colocalization of isotropic, fluorescent particles. *Histochem. Cell Biol.*, 139(3):391-402.

(5) Hakam, M.S., Miranda-Sayago, J.M., Hayrabedyan, S., Todorova, K. Barnea, E.R. and Fernandez, N. (2017). Preimplantation factor (PIF) promotes trophoblastic cells HLA-G and progesterone receptor expression and secretion. *Cellular Physiology & Biochemistry* 43:2277-2296.

(6) Roberto X. Calix, Sara Ornaghi, Jean H. Wilson, Nelson Fernández, Francois Vialard, Eytan R. Barnea and Michael J. Paidas (2017). Pre-implantation Factor and Endocrinology of Implantation and Establishment of Early Pregnancy: A Contemporary View. *Pediatric Endocrinology Reviews* 15 (No 2) 147-158.

CAR-T cell approaches to AML treatment

Yupo Ma[1,2]

1 – Department of Pathology, Stony Brook University, USA;
2 – Research and Development Division, iCell Gene Therapeutics LLC, Stony Brook, USA

Clinical trials with CD19 and BCMA-directed CARs have achieved unprecedented remission rates. However, translation of CAR therapy's success with B-cell and plasma cell malignancies to AML has not yet been accomplished. Translating CAR's success to AML requires a careful understanding of AML's unique characteristics. AML bears heterogenous target antigen expression on diverse myeloid population including on normal hematopoietic stem cells leading to off-target toxicity. Unlike B-cell and plasma cell malignancies, AML is uniquely challenging to treat due to the role of leukemic stem cells (LSCs). Since LSCs remain mostly in the quiescent phase of the cell cycle, chemotherapy directed against the rapidly dividing tumor populations leaves LSCs untouched. Most often this elusive LSC population comprises minimal residual disease (MRD) and is responsible for the inevitable relapse after AML treatment. Successful translation of CAR therapy to AML, to eliminate the disease, requires careful antigen selection that will enable the eradication of both bulk leukemic disease and leukemic stem cells.

We propose the use of a CLL1-CD33 compound CAR (cCAR) that will ablate both CLL1+ and CD33+ leukemic cells. CD33 are abundantly present on AML while CLL1 is overexpressed in leukemic stem cells in the patients who fail to achieve complete remission after induction chemotherapy. CAR targeting CLL1 may represent an effective means of eradicating minimal residual disease (MRD). However, both CD33 and CLL1 are also present on normal hematopoietic stem/progenitor cells, raising a question regarding potential myeloablation and a rescue allogeneic stem cell transplant. We have developed a Phase I clinical trial (Clinical#: NCT03795779) in the R/R AML patients using cCAR bearing two complete CLL1 and CD33 CAR constructs connected by a self-cleavage peptide. cCAR T cells were manufactured in a cGMP facility. The primary end point was to evaluate the safety of targeting CLL1 and CD33 antigens simultaneously, and the secondary end point is to explore the disease response and engraftment after the subsequent reduced intensity conditioning hematopoietic stem cell transplantation (HSCT). All patients selected for the study were transplant-eligible with an HSCT donor, yet unable to proceed due to residual disease. All patients received conditioning regimen of fludarabine and cyclophosphamide intravenously for three consecutive days with doses of 30 mg/m2/day and 300 mg/m2/day, respectively before the infusion of CAR-T cells. CAR-T cells were given by a dose escalation at 1-3X10^6/kg with a single or split dose. On disease reevaluation within 4 weeks post CAR-T cell infusion, 9 of 12 patients were MRD- by flow cytometry, and 3 of 12 had no response, one of which was $CD33^+$/CLL^-, indicating the importance of CLL1 target in the CAR-T treatment. The details of response, management, and toxicity will be discussed.

Conclusion: Our study indicates that CLL1-CD33 cCAR is a novel therapy with high efficacy and manageable toxicity in R/R AML patients. CLL-1-CD33 cCAR may provide a platform of reduced intensity con-

ditioning or nonmyeloablative conditioning HSCT for treating AML and other myeloid leukemias. On one hand, it can target bulky AML as well as leukemic stem cells. On the other hand, it can target normal hematopoietic stem cells, making it possible to reduce the intensity of conditioning of HSCT. This would reduce the organ toxicity as well as the incidence of GVHD, and consequently reduce the transplantation related mortality and the relapse related mortality.

Disclosures: Conflict-of-interest disclosure: Yupo Ma is the founder for iCell Therapeutics in the USA

Tumor-derived exosomes and their role in cancer progression and response to immunotherapy

Thresa L. Whiteside

Depts. of Pathology and Immunology, University of Pittsburgh School of Medicine and UPMC Hillman Cancer Center, Pittsburgh, PA 15213, USA

Emerging evidence emphasizes the important role extracellular vesicles (EVs) play in different physiological and pathological conditions, including cancer. Exosomes are a small (virus-size) subset of EVs that originate from the multivesicular bodies (MVBs) of parent cells. Exosomes carry a complex molecular cargo of proteins, lipids, glycans and nucleic acids derived from the endocytic compartment of parental cells. Serving as an intercellular communication system, exosomes deliver this cargo to target cells. Tumor-derived exosomes (TEX) maintain a cross talk between the tumor and various cells, including immunocytes, in the tumor microenvironment (TME) and are responsible for re-programming of the TME. TEX constitute a variable proportion of exosomes in plasma of cancer patients. Using immunocapture with antibodies to tumor-associated antigens (TAA), we separated TEX from non-TEX in plasma-derived exosomes of patients with melanoma or head and neck cancer. We compared molecular content of TEX with non-TEX by Western blots, flow cytometry and LC-MS/MS. The TEX cargo was enriched in immuno-suppressive proteins, and they suppressed functions of immune cells *in vitro* and *in vivo* in animal models of tumor growth. In contrast, non-TEX carried an excess of immunostimulatory proteins. The ratios of stimulatory to inhibitory proteins varied broadly among patients with cancer and correlated with disease activity and response to immune therapies. Studies of the quality and quantity of TEX molecular cargos underlie the concept of "liquid biopsy." Exosomes are emerging as a potentially useful diagnostic tool and a non-invasive predictor of disease progression, response to therapy and overall survival. In addition, circulating immunosuppressive TEX interfere with immune therapies, including the checkpoint inhibition. Silencing of TEX or reduction in their numbers in patients' plasma are novel therapeutic options for improving responsiveness of patients to immune therapies. Further, the role of TEX as diagnostic or prognostic biomarkers of cancer is under intensive investigation. There are many challenges to validation of their role as a liquid biopsy in cancer, including the existing controversy about their nomenclature and methods of exosome isolation. Nevertheless, newer approaches to characterization of circulating exosomes in patients with cancer offer hope for their future validation as cancer biomarkers and regulatory elements of the cancer-immune cell cross talk.

Immunopathogenesis of hypertension – from novel mechanisms to therapeutic targeting

Tomasz Guzik

Department of Internal and Agricultural Medicine, Jagiellonian University, Collegium Medicum
Institute of Cardiovascular and Medical Sciences, University of Glasgow, UK

Inflammation is an essential driver of cardiovascular disease. While understanding the role of the immune system is well developed in relation to atherosclerosis, much less is known about inflammation in hypertension. Several years ago, our group identified that T cells modulate the development of hypertension in several experimental models. These observations were confirmed in mice, rats, and more recently in human large-scale epidemiological and genetic studies. The mechanisms of T cell activation remain unclear but the generation of neoantigens through isolevuglandin (arachidonic acid metabolites) linked oxidative stress to immune responses in hypertension. However, bystander inflammatory activation of immune cells by cytokines is also taken into consideration. Hypertension prevalence is increased in multiple inflammatory diseases such as rheumatoid arthritis, SLE, or periodontitis. We have recently demonstrated a causal association between periodontitis and hypertension. Activated immune cells are targeted to perivascular adipose tissue and the kidneys where via effector cytokines such as IFN-g, IL-17, TNF-a they modify vascular and renal function. Mice lacking T cells, macrophages or any of the listed effector cytokines are protected from severe hypertension indicating possible therapeutic options related to immune and inflammatory targeting.

Progress in the production of therapeutic haematopoietic cells from pluripotent stem cells

Professor Lesley M. Forrester

Centre for Regenerative Medicine, Institute for Regeneration and Repair, University of Edinburgh

Cell therapies are currently used to treat many haematological diseases. These includes the transplantation of haematopoietic stem cell (HSC) that can reconstitution the entire haematopoietic system in the long term, transfusion of red blood cell (RBCs) or platelets as emergency short-term treatments and cells of the immune system that can fight infection and repair tissue. Limitations in supply and the risk of transmitting infection has prompted the design of protocols to produce these cell types from human pluripotent stem cells (hPSCs). Although significant progress has been made in the development of differentiation protocols that can successfully produce haematopoietic cells in vitro, it has proven really challenging to generate functional HSCs that can reconstitute the haematopoietic and some haematopoietic cell types do not appear to mature effectively. This plenary lecture will describe the strategies that we have used to address some of these problems. To address the problems associated with the production of HSCs, we carried out single cell RNA sequencing on haematopoietic stem and progenitor cells (HSPCs) derived from hPSCs in vitro and compared their transcriptional profile to HSPCs from the human fetal liver. We identified novel markers that will allow us to track the production of HSPCs in vitro and we used machine learning to discover molecular pathways that appear to be missing in hPSC-derived HSCs compared to their in vivo counterparts. This knowledge will now be used to design novel protocols for the production of functional HSCs. Attempts to generate RBCs from hPSC as an alternative to donor-derived blood transfusion have been hampered by the failure of erythroid cells to undergo the final maturation step and extrude their nuclei. This process occurs in vivo within erythroid islands (EI), where maturing erythroid cells surround a central macrophage that supports their maturation. To study the molecular processes involved in RBC maturation, we created an in vitro model of the EI niche using hPSC-derived macrophages. We discovered that the interaction between EI macrophages and maturing erythroid cells involve both secreted factors and cell-cell contact interactions and we are now studying how these factors can be used to improve the production of RBCs from hPSCs.

1. Adoptive Cell Therapy

Adoptive cell therapies as anticancer strategies

Radoslaw Zagozdzon

Department of Clinical Immunology, Medical University of Warsaw, Nowogrodzka 59 Str., 02-006 Warsaw, Poland

Recent advances in immunotherapy of cancer have provided evidence that a range of immune effector cells, such as cytotoxic T lymphocytes, natural killer cells or macrophages, can be successfully used for elimination of various types of cancer, once properly stimulated and/or engineered in the laboratory. Examples of such adoptive cell-based strategies are anticancer immunotherapies employing tumor-infiltrating lymphocytes, activated natural killer cells, engineered T cell receptor-expressing cells or chimeric antigen receptor-bearing cells. These therapies have already demonstrated success and have been approved in a range of hematologic malignancies, especially the ones of B-cell origin. However, in solid tumors cellular immunotherapies still face a number of substantial obstacles. These obstacles can be related, among others, to the choice of a target molecule, heterogeneity of cancer cells, immunoselectivity of the malignant tumor, as well as the metabolic and immune checkpoints utilized by cancer cells and the tumor microenvironment. There are issues with generalized toxicity of some adoptive therapies and an existing need for development of effective "off-the-shelf" types of cellular therapies, as most of the current ones are carried out in autologous settings. Nevertheless, multiple lines of evidence suggest that properly designed cellular therapies may in time become successful against a number cancer types and such studies are being carried out in a number of laboratories. Indeed, the adoptive cell therapies against cancer are constantly evolving and expanding, both alone or in combinations with other treatments, and are being evaluated in a variety of preclinical studies or clinical trials in cancer patients.

Teaching Tregs to respond to specific antigen

Dorota Iwaszkiewicz-Grzes[1,6,†], Mateusz Gliwinski[1,6,†], Anne Eugster[2], Magdalena Piotrowska[1], Andreas Dahl[3], Natalia Marek-Trzonkowska[4,5,6], Piotr Trzonkowski[1,6]

1 – Department of Medical Immunology, Medical University of Gdansk, Dębinki 7 Street, building 27, II floor, 80-952 Gdańsk, Poland; dorota.iwaszkiewicz@gumed.edu.pl
2 – Technische Universität Dresden, DFG-Center for Regenerative Therapies Dresden Cluster of Excellence, Fetscherstr. 105, 01307 Dresden, Germany
3 – Technische Universität Dresden, DRESDEN-concept Genome Center, Center for Molecular and Cellular Bioengineering (CMCB), Fetscherstr. 105, 01307 Dresden, Germany
4 – Laboratory of Immunoregulation and Cellular Therapies, Department of Family Medicine, Medical University of Gdańsk, Gdańsk, Poland
5 – International Centre for Cancer Vaccine Science, University of Gdańsk, Wita Stwosza 63, 80-308 Gdańsk, Poland
6 – Poltreg S.A., Trzy Lipy 3 Street, 80-172 Gdańsk, Poland

Background: T regulatory cells constitute about 1% of all peripheral blood lymphocytes, but they are instrumental in maintaining tolerance to self-tissues. Recently, Tregs has become used as a cellular drug. Clinical trials indicate that the therapy with this cells is safe and does not impair the immune response against foreign and dangerous antigens. Tregs isolated and expanded for cell therapy are polyspecific/polyclonal – (specific against many different antigen peptides), and thus their effectiveness to suppress in a tissue specific manner after administration is limited. Nevertheless, the efficacy of such a product can be increased by selecting antigen-specific Tregs which potentially migrate to the sites of antigen expression, where they selectively inhibit the activity of pathological effector cells with similar specificity. Here we describe a method that allows the in vitro preparation of antigen-specific T regulatory lymphocytes preparation for clinical use in the treatment of autoimmune diseases such as type 1 diabetes.

Methods: In our research Tregs were cultured with autologous monocytes loaded with a model peptide and then sorted into the cells recognizing the presented antigen and expanded. The specificity was verified with functional assays in which Tregs suppressed proliferation or IFNγ production of autologous T effector cells (polyclonal and antigen-specific) used as responders challenged with the model peptide. Finally, we analyzed clonotype distribution and TRAV gene usage in the specific Tregs.

Results: Tested method had a good yield and allowed us to obtain Tregs product enriched with specific subset as confirmed in the functional tests. The product consisted of many clones, nevertheless the content of these clones was different from that found in polyclonal or unspecific Tregs.

Conclusion: The presented technique might be used to generate populations of Tregs enriched with cells specific to any given peptide which can be used as cellular therapy medicinal product in antigen-targeted therapies.

This work has been supported by National Centre for Research and Development, Poland: LIDER/160/L-6/14/NCBR/2015, STRATEGMED1/233368/1/NCBR/2014 and POIR.01.01.01-00-0769/15-03. NMT was supported by the project „International Centre for Cancer Vaccine Science" that is carried out within the International Agendas Programme of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund.

Increased release of microparticles in patients with selected blood proliferative diseases

Mateusz Dziemiańczuk[1,2], Magdalena Bujno[1], Jarosław Piszcz[2], Anna Rogowska[1], Marzena Galar[2], Janusz Kłoczko[2], Piotr Radziwon[1,2]

1 – Regional Center for Blood Transfusion and Transfusion Medicine in Bialystok, Poland
2 – Department of Hematology with Vascular Diseases Division, Medical University of Bialystok, Poland

Introduction: Microparticles (MP) exhibit pro- and anticoagulant activity and are closely related to congenital platelet dysfunction, heparin-induced thrombocytopenia and deep vein thrombosis. Due to the increased MP concentration in the course of many diseases, it seems that they may have significant diagnostic potential.

Aim: Determination of the amount of released microparticles and selected markers of the haemostasis in patients with polycythemia (PV), essential thrombocythaemia (ET), chronic lymphocytic leukemia (CLL), acute myeloblastic leukemia (AML) and multiple myeloma (MM) before treatment.

Material and methods: The material for the study was venous blood collected from patients diagnosed with haematopoietic hyperplasia. The control group was material collected from healthy volunteers. Peripheral blood morphology, basic markers of the hemostasis system: PT, APTT, fibrinogen and d-dimer concentration were determined. Number of MP from blood cells and endothelium was determined using a flow cytometer using a mixture of labeled antibodies: CD235, CD45, CD42b and CD144. The content of thrombin-antithrombin (TAT), plasmin-anti-plasmin (PAP) and prothrombin 1 + 2 (F1 + 2) fragments was determined by ELISA.

Results: An increase in the number of all examined microparticle types was observed. In the case of PV, a significantly increased number of erythrocyte-derived microparticles was observed, while in ET there was a dominant increase in the percentage of platelet-derived microparticles. For CLL, AML and MM, an increase in microparticles from all cells was observed. In PV, APTT prolongation, fibrinogen and d-dimer concentration are noticeable. In the course of ET, PT and APTT were prolonged, and elevated fibrinogen concentration. An increase in fibrinogen and d-dimers was observed in the case of CLL. In AML, PT and APTT lengthening as well as increased d-dimer concentration are important. An increase in coagulation times and an increase in the concentration of fibrinogen and d-dimers was observed in the course of MM. The content of TAT complexes was higher in patients diagnosed with PV and ET, the PAP content was increased in patients with PV, CLL, MM and the content of F1 + 2 was lower than in the control group in the course of ET and AML.

Conclusions: Blood proliferative diseases contribute to the release of microparticles from blood cells and endothelium and changes in hemostasis system. The concentration of MP correlates with different markers of hemostasis, dependent at least partially on the underlying disease. Future research is needed on microvesicles as biological targets for the diagnosis, prognosis and possible therapy of proliferative diseases.

Key words: microparticles, blood proliferative diseases, flow cytometry

2. Advances and challenges of immunogenetics towards personalized therapy

Next Generation Sequencing technology in HLA transplantation diagnostics

Christina E.M. Voorter

Department of Transplantation Immunology, Maastricht University Medical Center, Maastricht, The Netherlands

For stem cell- as well as solid organ transplantation, matching of HLA between patient and donor is important for survival results. Although more and more the standard, HLA matching based on High resolution HLA typing becomes more and more challenging due to the steadily increasing numbers of HLA alleles identified. Sanger sequencing has been the gold standard to perform High Resolution Typing for many years, but heterozygous sequencing has resulted in genotypic ambiguities in >70% of the samples at the end of 2014. The development of next generation sequencing (NGS) approaches can largely overcome this problem. At the moment, three types of NGS approaches are available for sequencing of HLA: the so called second, third and fourth generation sequencing methods. For second generation sequencing both Illumina and Ion Torrent are the preferred machines for HLA typing. With this method fragmentation of the PCR products is needed and this can result in phasing problems when adjacent polymorphic positions are located too far from each other to enable cis-trans definition. With third generation sequencing using an expensive PacBio machine, a single molecule with lengths up to 100 kb can be sequenced without any phasing problems. With the fourth generation, the MinION Nanopore technology, also a single molecule with lengths up to 2000 kb can be measured. In contrast to second and third generation, Nanopore sequencing is not performed by synthesis of a new strand, but by passing the DNA molecule through a nanopore, changing the electric current across the pore. All three NGS methods have some problems with the correct analysis of large homopolymers and repeat sequences that are present in the introns. Furthermore, prerequisite for High Resolution Typing is the exclusion of null alleles, wherever the polymorphism is located. This implicates that NGS is not identical to high resolution typing, because it is depending on the part of the HLA gene that is investigated. This turns out to be especially a problem for the HLA class II gene DQB1. Overall, NGS is a suitable method for high resolution HLA typing, but for the exclusion of null alleles additional exon and/or intron sequences might be needed.

Haploidentical stem cell transplantation in children with non-malignant diseases

J. Toporski, CJ. Pronk, L. Krol, J. Dykes, D. Turkiewicz.

Skåne University Hospital, Lund, Sweden

Allogeneic hematopoietic stem cell transplantation (HSCT) has been used as a replacement therapy for patients with bone marrow failure/hemoglobinopathies (BMF/H), immunodeficiencies (ID) and a variety of inherited metabolic disorders (IMD). If an HLA-matched donor is not available, HSCT from a haploidentical family donor (haploSCT) may be considered. We report a series of 16 children (BMF/H 5, ID 7, IMD 4) who received T cell depleted haploSCT between 2008 – 2021 (17 transplants).

In 12 cases the indication was primary disease, in 5 cases haploSCT was done as a rescue from the graft failure after previous HSCT (UCB 2, MUD 2, haploSCT 1). All children were transplanted with T-cell depleted (immunomagnetic depletion) parental PBSC graft. Conditioning included serotherapy, Fludarabine based myeloablation and Rituximab as in vivo B cell depletion. Three children with SCID did not received any conditioning. Apart from T-cell depletion/serotherapy no GvHD prophylaxis was used. Thirteen children achieved full and sustained hematopoiesis with ANC >500/μL at day +13. One child rejected the graft and was successfully regrafted. Two children died before engraftment.

In all children but two stable donor chimerism was achieved in all cell lines. In those transplanted without conditioning T lymphocytes are shown to be 100 % while other cells are of recipient origin.

Four children had aGvHD ≥2 at the time of engraftment (3 limited to skin, 1 skin + liver) and responded well to a short steroid treatment.

The most common infectious complication was reactivation of AdV (adenoviremia), in one child progressing to symptomatic disease. For this reason, 4 children received CD45RA depleted donor lymphocyte infusion. No GvHD was seen after DLI and 3 children cleared viremia shortly after DLI.

As for now 12 out of 16 children are alive and well, with no cGvHD, 3 mos to 13 yr (median 6,1 yr) after transplantation. Two patients with BMF died early after grafting – day +9 and +16 due to MOF following septicemia. Another 2 patients died day +111 and 132 due to MOF triggered by AdV and pulmonary alveolar hemorrhage respectively.

These data indicate that in the absence of a suitable HLA-identical donor, haploidentical HSCT may be a viable option for patients with life-threatening disease and urgent need of HSCT.

Adoptive immunotherapy with Natural Killer cells – past, present and future

Bartosz Grzywacz

Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN, USA

Immunotherapy of cancer has become a viable treatment option in the recent years, one of the therapeutic applications of the immune system is adoptive immunotherapy with Natural Killer cells. There are many aspects of adoptive immunotherapy with NK cells which are under active development in the recent years. They will be divided into four areas:

1) Activation and expansion of NK cells ex vivo and in vivo – cytokines and growth factors
2) Choices and modifications of NK cells to achieve highest number and highest activity
3) NK cell "engagers" to bring NK cells closer to the target and activate them
4) Factors of the host and the tumor cells which influence effectiveness of NK cells and how to modify them

The field of NK cell immunotherapy has made great strides in the areas 1-3 with some of these developments following in the footsteps of T cell immunotherapy, for example the application of Chimeric Antigen Receptors (CAR). In other aspects NK cells have bypassed the T cell immunotherapy due to the relative ease of their generation from hematopoietic stem cells and from induce pluripotent stem cells. The fourth area – identification of host and tumor factors requires more work, since little is known about the factors affecting the trafficking, expansion and persistence of NK cells after the infusion. We present results of our research showing that depletion of Tregs using immunotoxin and low-dose total body irradiation improve persistence of NK cells in the bone marrow after adoptive immunotherapy for acute myeloid leukemia. These are very exciting times in NK cell immunotherapy and there is great hope in application of these innate immune cells to the treatment of hematologic and solid tumors.

20 years of Wrocław HLA External Proficiency Testing

Katarzyna Bogunia-Kubik

Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland

Proficiency testing plays an important role in quality management systems implemented in diagnostic laboratories. They allows to verify practical skills of laboratory staff and extend the range of methods used, eliminate the sources of potential errors, and identify the directions for further development. This also applies to histocompatibility and immunogenetics laboratories, for which participation in one or more external proficiency testing (EPT) schemes is often mandatory to meet national standards and accreditation requirements, such as those of the European Federation for Immunogenetics (EFI). Our Wrocław EPT, serving EFI Region 5 (Central Europe), is one of 16 local EPT providers covering 9 EFI EPT Regions.

HLA Proficiency Testing for Central and East Europe (formerly Proficiency Testing of HLA class I Typing for Central and East Europe) was initiated by Professor A. Lange in 1999 and is continued under the auspices of the Polish Society for Immunogenetics. This year, the event will be held for the 28th time. To date, 67 laboratories from 15 countries, also from outside Central and Eastern Europe, have participated in our EPT.

During more than 20 years of Wrocław EPT activity, the scheme has evolved to meet the requirements of the growing number of participants and to include some technological novelties. Currently, it covers HLA class I serological typing and HLA class I and class II DNA typing at both low and high resolution levels. Last year, molecular typing was expanded from 7 to 11 HLA loci, which includes HLA class I (A, B, C) and class II (DRB1, DRB3, DRB4 and DRB5; DQA1 and DQB1; DPA1 and DPB1).

The organization of EPTs requires much effort, starting with the registration of participants, the preparation of samples and their shipment, followed by the collection and evaluation of typing results, and issuing of certificates. The undisputed benefit of EPTs is the improvement in quality of histocompatibility testing, which translates directly into more effective and reliable donor-recipient matching, and thus better transplantation outcomes.

Functional evolution of NK cells

Jacek Nowak

Department of Immunogenetics, Institute of Hematology and Transfusion Medicine, Warsaw

NK cells play an important role in anti-tumor, antiviral and reproductive immunity. The lecture presents the mechanisms of stochastic expression of NK cell receptors and the NK cell recognition of a "healthy" self and transformed target cells. The relationship between education phenomena and NK cell effector response is emphasized. The role of inhibitory KIR receptors in increasing the education of NK cells (arming) and the opposite role of activating KIR receptors (disarming) have been pointed out. NK cell immunophenotype differences are shown among unlicensed/naive NK cells [low granzyme B, $CD56^{bright}$, NKG2A +, iKIR (-), active proliferation] and licensed/adaptive/mature NK cells [NKG2A (-), iKIR +, CD57 +, granzyme B++, no dividing]. The implications of the existence of a functional phenotype of licensed NK cells (Ca^{2+} release, CD107a, IFN gamma and TNF secretion, NK cell conjugation with target cells in vitro and in vivo) are discussed. The directions of clinical application of the research on KIR receptors and ligands as well as lysosomal activity of NK cells are presented. Routine cell therapy is discussed, in which the results of own research on the high activity of licensed NK cells in reducing haematological cancer relapse (graft versus leukemia) and elimination of EBV infections in hematopoietic stem cell transplant recipients are presented. A hypothesis on the reasons for the lack of beneficial effects of licensed NK cells in CMV infections after hematopoietic cells transplantation is presented. In terms of future therapies, the idea of "off-the-shelf" cell therapies is presented: i) preparation of synthetic NK cells with increased lysosomal activity (iPSC-derived NK cells), and ii) selective expansion of adaptive NK cells from "super donors".

The epigenetic landscape in regulatory and conventional T cells upon antigen stimulation

Magdalena Piotrowska[1], Dorota Iwaszkiewicz-Grzes[1], Mateusz Gliwinski[1], Piotr Trzonkowski[1]

1 – Department of Medical Immunology, Medical University of Gdansk

Background: Regulatory T cells (Tregs), which main function is blocking T effector cells (Teffs), are used in many clinical trials. Nowadays polyclonal Tregs are used to treat autoimmune diseases, e.g. type 1 diabetes (T1D). Nevertheless, the possibility to use antigen-specific (Ag-spec) Tregs towards particular antigen seems to be a promising tool but cells expansion should preserve their functional stability and phenotype. In this study we aim to determinate epigenetic changes that occurs in Ag-spec Tregs and Teffs during cell culture.

Methods: Tregs ($CD4+CD25^{high}CD127^{low}$) and Teffs ($CD4+CD25^{low}CD127^{high}$) from human buffy coats obtained from anonymous healthy donors (Regional Blood Bank in Gdansk) were isolated and characterized using flow cytometry. Cells were subjected into TCR stimulation by autologous monocytes presenting antigens important in T1D: whole insulin or insulin β chain peptide 9-23. After expansion, cells were labelled, using cell proliferation kit, and sorted into specific ($SPEC_{INS/B:9-23}$) and unspecific ($UNSPEC_{INS/B:9-23}$) populations. Histone H3 post-translational modifications (PTMs) and global DNA methylation were measured using the colorimetric assay. The methylation level of Treg-Specific Demethylated Region (TSDR) was analysed by quantitative methylation-specific PCR (Q-MSP). Additionally, expression of Tregs-related genes was assessed using the real-time PCR technique.

Results: All Tregs had high expression of genes required for their function, with the superiority of Tregs $SPEC_{B:9-23}$ over insulin-specific Tregs. Both global methylation and TSDR were lower in Tregs $SPEC_{B:9-23}$ in comparison to Tregs $SPEC_{INS}$. A characteristic H3 modification pattern was observed in particular Treg/Teff pairs, where low level of PTMs in one subset from the pair was connected with high level in the other. Tregs $SPEC_{INS/B:9-23}$ were abundant in activating PTMs: H3K18ac, H3K9me1 and, H3K36me2, while H3K14ac and H3K27me1 were modified only in Tregs $SPEC_{B:9-23.}$

Conclusions: Stimulation with monocytes presenting antigens exerts epigenetic changes in Tregs. Ag-spec Tregs maintain their suppressive phenotype and remain stable during cell culture. Insulin β chain peptide 9-23 promotes Tregs-oriented epigenetic changes, whilst insulin stimulation is less understandable.

Association of IL-13 polymorphism with response to TNF inhibitors in rheumatoid arthritis patients

Sylwia Biały[1], Milena Iwaszko[1], Jerzy Świerkot[2], Katarzyna Kolossa[3], Sławomir Jeka[3,4], Katarzyna Bogunia-Kubik[1]

1 – Laboratory of Clinical Immunogenetics and Pharmacogenetics, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland
2 – Department of Rheumatology and Internal Medicine, Wroclaw Medical University, Wroclaw, Poland
3 – Department of Rheumatology and Connective Tissue Diseases, Jan Biziel University Hospital No. 2, Bydgoszcz, Poland
4 – Ludwik Rydygier Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun, Poland

Interleukin-13 (IL-13) is a crucial cytokine of Th2 cell-mediated immune responses. IL-13 has important immunomodulatory activities and exerts influence on wide variety of immune cells. This cytokine is a key mediator of allergic inflammation. Recent studies have implicated role of IL-13 in pathogenesis of autoimmune diseases, such as rheumatoid arthritis (RA). The aim of this study was to evaluate a plausible role of polymorphism within IL-13 gene in RA development and efficacy of anti-TNF therapy.

A total of 466 RA patients receiving anti-TNF treatment and 229 healthy controls were enrolled to the study. Genotyping for IL-13 rs20541 was performed using a polymerase chain reaction (PCR) amplification employing LightSNiP assays. Clinical response was evaluated according to the European League Against Rheumatism (EULAR) criteria at 12th and 24th week after initiation of the therapy.

The IL-13 rs20541 was significantly associated with response after 12 weeks of anti-TNF treatment. Presence of IL-13 rs20541 A allele among patients significantly correlated with better outcome of therapy (p=0.035). Also patients carrying heterozygous IL-13 rs20541 AG genotype achieved good response to anti-TNF agents more frequently than patients with other genotypes (p=0.029). On the other hand, inefficiency of anti-TNF therapy was more frequently observed in patients with GG genotype as compared to patients bearing other genotypes (p=0.019). No association was observed between IL-13 rs20541 polymorphism and predisposition to RA development. These results suggest that IL-13 polymorphism may influence response to therapy with TNF inhibitors in patients with RA.

This work was supported by the National Centre of Science (Poland) – grant no. 2016/21/B/NZ5/01901.

TERT genetic variability and telomere length as factors affecting survival of acute myeloid leukaemia patients

Marta Dratwa[1], Barbara Wysoczanska[1], Aleksandra Butrym[2], Grzegorz Mazur[3], Katarzyna Bogunia-Kubik[1]

1 – Laboratory of Clinical Immunogenetics and Pharmacogenetics, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland;
2 – Department of Cancer Prevention and Therapy, Wroclaw Medical University, Wroclaw, Poland;
3 – Department of Internal, Occupational Diseases, Hypertension and Clinical Oncology, Wroclaw Medical University, Wroclaw, Poland

Telomerase reverse transcriptase (*TERT)* is the catalytic subunit of the telomerase complex that maintains genomic integrity via telomere length (TL) and plays an important role in leukemia's development. The present study aimed to find out whether TL and *TERT* gene polymorphisms may contribute to disease risk or progression.

For these purpose TL and *TERT* (rs2736100, C>A, and rs2853669, C>T) variants were assessed in 95 AML patients and related with clinical data, including *FLT3*-ITD and *NPM1* mutation status and survival. In addition, 133 healthy individuals served as controls. TL was assessed using qPCR assay kit (ScienCell's Absolute Human Telomere Length [AHTLQ] Quantification qPCR Assay Kit Carlsbad, CA, USA). *TERT* polymorphic variants were detected with the use of LightSNiP typing assay (TIB-Molbiol, Berlin, Germany).

TL was negatively correlated with patients' age in healthy subjects ($p<0.0001$), in opposite to AML cases ($p=0.029$). Patients with *TERTp CC* genotype were characterized with shorter overall survival (OS) than patients carrying the *T* allele ($p=0.028$). Moreover, patients below 61 years (mean age) lived longer than older patients ($p=0.007$) and possessed shorter TL as compared to patients > 61 years of age ($p=0.046$). We observed, that among AML patients <61 years of age, those carrying the *FLT3*-ITD mutation had significantly lower TL than patients lacking this mutation ($p=0.003$). Moreover, the presence of *FLT3*-ITD mutation in this group of younger AML patients was found to be associated with significantly worse OS ($p=0.038$). We observed that patients with favourable risk classification (*NPM1*$^+$ and *FLT3*-ITD$^-$) were characterized with longer TL than AML patients with adverse risk, $p=0.019$). We also observed that patients with intermediate risk classification (related to mutual presence or absence of *FLT3*-ITD and *NPM1* mutations) had longer TL as compared to the adverse group, $p=0.013$). Moreover, a multivariate Cox regression analysis proved that patients heterozygous for rs2853669 ($p=0.055$) and the presence of *NPM1* mutation ($p=0.080$) showed a positive association with overall survival, while higher WBC count ($p=0.0002$) and more advanced patients age ($p=0.053$) showed an adverse effect.

In conclusion, OS of AML patients appears to be affected by TL and *TERT* variability in addition to other well-established factors such as age or *FLT3*-ITD and *NPM1* mutation status.

This work was supported by the TARGETTELO project No. STRATEGMED3/306853 from the National Centre for Research and Development, Warsaw, Poland.

Genotyping of NKG2D and MICA variants in a paediatric cohort of patients after HSCT – preliminary results

Jagoda Siemaszko[1], Milena Iwaszko[1], Blanka Rybka[2],
Renata Ryczan-Krawczyk[2], Marek Ussowicz[2], Katarzyna Bogunia-Kubik[1]

1 – Laboratory of Clinical Immunogenetics and Pharmacogenetics, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland
2 – Department and Clinic of Paediatric Bone Marrow Transplantation, Oncology and Haematology, Wroclaw Medical University, Wrocław, Poland

Natural killer (NK) cells play a significant role in patients after allogeneic haematopoietic stem cell transplantation (HSCT), being the first lymphocyte subset to appear in the peripheral blood after transplantation. They play an important role in the immune response against cancer and viral infections. NKG2D is one of the activating receptors of NK cells. Its interaction with the non-classical MICA molecule is crucial for the proper functionality of the NK cells. The aim of this study was (i) to evaluate whether genetic variants in genes coding for NKG2D and MICA could be used as predictive biomarkers for transplantation outcomes and (ii) to determinate differences in the analysed polymorphisms between patients and controls.

For this purpose, one hundred DNA samples isolated from children with haematological disorders and approved for HSCT were analysed. Additionally, 236 healthy control subjects were included. Genotyping for NKG2D (rs1049174, rs1154831) and MICA (rs1051792, rs1063635) alleles was performed using LightSNiP assays.

Patients and controls differed with respect to the presence of homozygosity for the NKG2D coding gene, rs1049174 *G* allele, which was more frequently detected among patients (RR=5.442, $p<0.001$). This rs1049174 *G* allele also appears to predominate among patients who developed CMV reactivation after transplantation (37% vs 11%, ns). Patients carrying the *MICA* rs1051792 *GG* genotype were less likely to develop acute GvHD grade 2-4 (79% vs 96%, $p=0.045$). Furthermore, none of the patients with chronic GvHD carried the *MICA* rs1063635 *AA* genotype, which was present in 24% of patients without symptoms of chronic GvHD. Moreover, the presence of the rs1063635 *A* allele was found to be associated with better patients' overall survival. This genetic variant was not present in any of the patients who died after transplantation, while it was detected in 25% of transplant survivors ($p=0.063$).

These preliminary results imply that *NKG2D* and *MICA* gene polymorphisms may be of predictive value for HSCT patients. However, they require validation and confirmation in more extensive studies involving larger patient cohorts.

This work was supported by the project No. 2018/31/B/NZ2/03065 from the National Science Centre (Poland).

3. Allergen Immunotherapy and Biological Treatment

From personalized to precision medicine – advances in allergy treatment

Marek Jutel

Professor and Chair, Department of Clinical Immunology, Wroclaw Medical University, Wroclaw, Poland

Allergen-specific immunotherapy (AIT) represents the only curative treatment for allergic diseases. Marked differences between AIT products, regulation, and standard of care exist between the United States and Europe. Intense research has been focused on elucidation of mechanisms of the AIT–induced tolerance. Recently the major efforts are directed towards the finding of the plausible biomarkers. This is in line with the precision/personalized medicine approach aiming at the prospective stratification of the potential responders and non-responders to the treatment as well as identification of the best laboratory endpoints correlating with the clinical response. These biomarkers should be considered in terms of the rapid/short term desensitization as well as long medium- and term tolerance. Until now the basophil activation markers – formation of sLTs or intracellular expression of diamineoxidase in basophils as well as histamine associated signal – H1R/H2R expression ratio in DC, T cells are the most promising biomarkers. Regarding the medium- and long-term tolerance the frequency of allergen specific T- and B-reg, levels of IL-35 and numbers of iTR35 cells or induction of immune-reactive and functional IgG4 antibodies provide the best option. Among *in vivo* approaches the clinical assessment in the environmental exposure chamber seems to be the best approach, however the standardization between different chambers seems to be the major issue at present. Late phase response (LPR) in the intracutaneous allergen testing has also been validated as a reliable *in vivo* biomarker. Currently, the standard of care involves SCIT and SLIT with allergen extracts. Over the last decade a number of innovative approaches failed either in phase II trials – recombinant B cell -epitopes of grass pollen, immunogenic peptides of grass pollen, recombinant Bet v 1 in SLIT-tablet for birch pollen allergy, toll-like receptor signal-based vaccines for ragweed or in phase III studies – hypoallergenic recombinant Bet v 1 in SCIT, recombinant 5 allergen (Phl p 1, 2, 5a, 5b, 6) cocktail in SCIT for grass pollen allergy, synthetic peptides of Fel d 1 in cat allergy or allergen peptide hydrolysates in grass pollen allergy. The innovative products currently under investigation include allergen-specific blocking IgG, plasmid DNA or hypoallergenic recombinant allergens. Adjuvants and new formulas also provide a promising option – nanoparticles, fusions proteins, unspecific immune stimulators (virus-like particles, liposomes, polysaccharide polymers. Novel routes of application (intralymphatic, epicutaneous, intradermal or buccal) are also under investigation. Thus, AIT is constantly evolving towards improved products and procedures and we are going to witness major developments over the next decades.

Jutel M et al. International consensus on allergy immunotherapy. J Allergy Clin Immunol. 2015 Sep;136(3):556-68.

Bousquet J, Jutel M et al. The Role of Mobile Health Technologies in Stratifying Patients for AIT and Its Cessation: The ARIA-EAACI Perspective. J Allergy Clin Immunol Pract. 2021 May;9(5):1805-1812.

The epidemic of allergic diseases – can we stop it?

Bolesław Samoliński

Warsaw Medical University

Allergy is a very common disease. Almost 40% of the Polish population, similar to European and US, suffers from nasal or asthma or atopic dermatitis together. But from the epidemiological point of view, the most important problem is the growing data on the occurrence of allergies almost in all well-developed countries. Over the past 100 years, allergy has increased over 13 times in Switzerland. The reason for this is still tested. Currently, the following causes responsible for the high incidence of allergies are recognized:

1. Hygienic hypothesis
2. Air pollution
3. A bad diet leading to overweight
4. Tobacco smoke
5. The use of antibiotics.

The mechanism of influence of all these factors for allergy is discussed in a lecture, especially because allergic diseases are a health problem and leads to civilized diseases in older people and probably premature death. The only way to stop the allergy epidemic is to change the lifestyle from the earliest period, improve the diet, reduce air pollution, smoking and excessively frequent use of antibiotics

Biologics and Biosimilars

Karina Jahnz-Różyk

Department of Internal Medicine, Pneumonology, Allergology and Clinical Immunology Military Institute of Medicine, Warsaw, Poland

Biological treatment is one of the greatest achievements of modern medicine. We owe it to the development of biotechnology, and the first biological drug was registered by the European Medicines Agency in 1995.

Newer generations of biological drugs, which include monoclonal antibodies (Mab) and fusion proteins (FP), were approved later – in 1997, rituximab and in 1998, infliximab. With the technological progress and the loss of patent protection by original drugs, biosimilars began to appear on the pharmaceutical market.

The first biosimilar drug – human growth hormone – appeared in 2006, and the first Mab (infliximab) in 2014. Other biosimilars – adalimumab, etanerecept and rituximab were registered in 2017.

The lecture will show the following issues:

1. Differences of biological and biosimilar drugs – their structure, nomenclature, production steps, pharmacokinetics, mechanism of action
2. Application in various medical areas
3. Selected aspects of biological therapies in allergic diseases
4. Safety of biological therapy
5. Administrative regulations – EMA v.s FDA
6. Access to biological treatment in Poland

Current concepts and future directions in biological therapy for allergy and asthma

Maciej Kupczyk

Dept. of Internal Medicine, Asthma and Allergy, Medical University of Lodz, Poland

Severe allergies and asthma represent a significant burden for the patient, his family and the health care system. This is due to the severity of the symptoms, drug costs, significant impairment of daily activities, quality of life and limitation in the professional work. Biological therapy represents the breakthrough in the management of spectrum of various diseases. Current concepts in biological therapy in allergies and asthma, new indications for the "old" molecules and future directions, including some pitfalls and dead-ends will be discussed. In asthma management, in case of ineffectiveness of the 4th step of GINA treatment, the patient should be referred to a specialist center to consider the additional treatment, including among others anti-IgE (omalizumab), anti-Il-5 (mepolizumab), or antibody against the α-subunit of receptor for Il-5 (benralizumab). Biological drugs are available in Poland as part of the therapeutic program for the treatment of severe asthma. New drugs including dupilumab (binding to the alpha subunit of the interleukin-4 receptor (IL-4Rα) blocking IL-4 and IL-13 pathways) and tezepelumab (which blocks thymic stromal lymphopoietin TSLP pathway) should be available soon. New indications for the "old"molecules comprise: omalizumab in sinusitis with nasal polyps and chronic spontaneous urticaria (CSU), mepolizumab for nasal polyps, hypereosinophilic syndrome, eosinophilic esophagitis and eosinophilic phenotype of chronic obstructive pulmonary disease (COPD). Preliminary studies proved high clinical efficacy of ligelizumab in the management of CSU and masitinib, which is the first-in-class oral tyrosine kinase inhibitor, in severe asthma. Several biologics have been studied in food allergy. An interesting approach, especially in atopic dermatitis, is represented by drugs directed against Janus kinase (JAK) and mitogen activated protein kinase (MAPK) including, but not limited to Abrocitinib, Baricitinib, Upadacitinib, Tofacitinib, Ruxolitinib, Delgocitinib, Cerdulacitinib, Gusacitinib. Extensive studies of biologics, despite some pitfalls and dead-ends in some projects, will introduce new molecules into our clinical practice with high effectiveness and optimal safety profile in the management of several allergic diseases and asthma.

Structural similarities of allergens. Implication for allergen immunotherapy

Krzysztof Kowal

Department of Experimental Allergology and Immunology, Medical University of Bialystok

Allergens originate from many allergen sources including a variety of animal and plant species. However, allergens belong to a relatively few of the identified protein families. Interestingly, allergens belonging to the same protein family share some sequence and structural similarities. Binding of allergen by immunoglobulin E (IgE) is crucial for triggering mast cell-dependent allergic reaction. Immunoglobulins, including IgE recognize 3D structure of antigenic epitopes. When structural similarity between allergens is high IgE fails to discriminate between the original sensitizer and other similar proteins. This, in turn leads to cross-reactivity to allergens derived even from phylogenetically distant allergenic sources. Getting to know the structural similarities between individual allergens should pave a way for better diagnostic methods and invention of more effective and simpler approaches to allergen immunotherapy.

Casein-specific miRNA-150 extracellular vesicles inhibit inflammatory reaction in mouse model of delayed-type hypersensitivity to casein

Magdalena Wąsik, Katarzyna Nazimek, Krzysztof Bryniarski

Jagiellonian University Medical College, Faculty of Medicine, Department of Immunology

Background: Suppressive extracellular vesicles (EVs) carrying miRNA-150 were proved to inhibit cell-mediated hypersensitivity reactions to haptens. These EVs, a product of CD8+ T cells, can act antigen-specifically, if coated with IgM light chains, secreted by B1a cells. We sought to test the potential therapeutic use of antigen-specific EVs in new mouse model of delayed-type hypersensitivity (DTH) to milk protein, such as casein.

Methods: Animals were intradermally injected with casein antigen and after 4 days effector cells were collected from spleens and lymph nodes. Tolerance was induced by intravenous injection of mouse erythrocytes conjugated with casein antigen and following immunization to casein. Regulatory cells were collected from spleens and lymph nodes of tolerized animals and cultured for 48h. EVs were harvested from the culture supernatant by methods of ultrafiltration and ultracentrifugation. In some experiments EVs were treated with inhibitor of miRNA-150 or separated by antigen-affinity chromatography. Effector cells were incubated with acquired EVs prior to adoptive transfer to naive recipients. EVs were also administered to the animals in active inflammation intradermally, intraperitoneally, intravenously or per os.

Results: Inflammatory reaction was effectively suppressed by antigen-specific EVs both, in transfer to naive recipients and in active inflammation. Oral administration of suppressive EVs induced the highest level of suppression. Suppressive capacity of EVs was completely reduced after miRNA-150 inhibition. EVs separated on casein column were proved to act casein-specifically in criss-cross experiments.

Conclusions: We confirmed that miRNA-150 EVs acquired from animals tolerized to food protein, such as casein, can antigen-specifically suppress DTH reaction. A test of therapeutic use of EVs proves that oral administration, which is also the natural route of immunization with the food allergens, is the most effective.

Study supported by Polish Ministry of Science and Higher Education (N41/DBS/000187).

4. Allergology

Allergic march

Marek Kulus

Klinika Pneumonologii i Alergologii Wieku Dziecięcego, Warszawski Uniwersytet Medyczny

Allergic march is the term describing typical sequence of allergic sensitization with parallel manifestation of clinical symptoms. The development of allergic diseases is the result of complex interactions between genetic predisposition and environmental factors. According to this hypothesis atopic dermatitis (AD) precede symptoms of food allergy and respiratory manifestation. Fouchard in 1973 as the first scientist described this condition. Cohort studies confirm the increased risk of asthma and allergic rhinitis in children with early manifestation of AD. Some of clinical conditions persist while others diminish or disappear completely. The pathogenesis of allergic march is still unclear and widely discussed. The theory indicating the dysfunction of the epidermal barrier as the primer of allergic sensitization to both food and airborne allergens seems to be most attractive nowadays. However, the classic allergic march during the past two, three decades seems to be getting less frequent. At the same time, we observe increase incidence of food allergy in general population. There are also data showing that 'reverse' atopic march in which the symptoms of allergic rhinitis and bronchial asthma can precede the development of AD. An alternative hypothesis suggests that allergic march is only the coincidence of atopic diseases.

The most relevant question is whether we can stop the atopic march. In this lecture author summarizes the results of available studies on the primary and secondary prevention of atopic diseases. Although numerous studies have already been performed the problem of prevention of atopic march remains unsolved.

Mutual relantionship between immunity and coagulation in urticaria and angioedema

Barbara Rogala

Uniwersyteckie Centrum Kliniczne, Śląski Uniwersytet Medyczny, Katowice

Chronic urtcaria (CU) is a common skin disease. It severely affects patients'quality of life. Infections, drugs, autoimmune, malignant diseases and psychological conditions are recognized relevant to etiology of CU. However no etiological factors are detected in the majority of patients. CU and urticaria associated angioedema is regarded as autoimmune disorder. Activation of the coagulation cascade is also proposed as a patomechanism of urticaria. The involvement of the coagulation system/fibrinolysis and non-infections inflammatory factors is confirmed in CU. The interplay between inflammation and coagulation plays the role in CU phenomenon. It might be implicated in the pathogenesis of the disease and may be associated with the risk of cardiovascular disorders in patients suffering from CU.

Which immunological tests are reliable in diagnosis of food allergy?

Katarzyna Napiórkowska-Baran, Zbigniew Bartuzi

Department of Allergology, Clinical Immunology and Internal Diseases, Nicolaus Copernicus University in Toruń Ludwik Rydygier Collegium Medicum in Bydgoszcz, Bydgoszcz, Poland

Food allergy has been estimated to affect nearly 2 to 5% of adults, compared with 6–8% of children. It is the third most common after allergy to pollen and house dust mites. The incidence is steadily increasing.

Allergological diagnostics is one of the most difficult compared to other specializations, e.g. due to possible unusual symptoms (e.g. headache, otitis media, sinusitis, pharyngitis, constipation, acute pancreatitis, Kounis syndrome), cross reaction, hidden allergens, delayed reactions (symptoms after 48 – 72 hours), IgE-dependent and Ig-E independent reactions, similarities in symptoms of other pathologies (lactose intolerance, SIBO, pseudoallergic reactions: exogenous histamine, biogenic amines), cofactors or multitude of food additives. Unfortunately, all of the available tests are not characterized by 100% sensitivity and specificity.

The study presents commonly available diagnostic methods of food allergy (skin tests, total IgE, specific IgE, patch tests and methods available only in reference centers (molecular diagnostics, basophil activation test, oral food challenges). Their advantages and limitations, sensitivity, specificity and factors that may affect both false positive and false negative test results are discussed. Tests that are not relevant to the diagnosis of food allergy were also mentioned.

Due to the fact that commonly available diagnostic methods allow to diagnose practically only IgE-dependent allergy, the problem of allergological diagnostics in the case of suspected disorders of antibody production was raised.

The problems discussed in the presentation are the result of the authors' experience, who deal with the diagnosis of food allergies on a daily basis.

Potential pro-inflammatory properties of *Tranzschelia pruni-spinosae* and *Phragmidium rubi-idaei*

Monika Sztandera-Tymoczek[1], Aleksandra Ziomek[1],
Urszula Świderska-Burek[2], Agnieszka Szuster-Ciesielska[1]

1 – Department of Virology and Immunology, Maria Curie-Skłodowska University
2 – Department of Botany, Mycology and Ecology – Institute of Biological Sciences, Maria Curie-Skłodowska University

Introduction: Allergic diseases are classified as civilization threats in the 21st century. An allergy is a pathological reaction of the immune system in response to contact with common antigens (allergens), including inhaled fungal spores. The most important allergenic fungi belong to the genera *Alternaria*, *Aspergillus*, and *Cladosporium*. Unfortunately, currently the cause of an existing allergy cannot always be identified, which may indicate sensitization to other fungal allergens. A potential source of allergens may be phytopathogenic microfungi, which include the representatives of plant rust – *Tranzschelia pruni-spinosae* and *Phragmidium rubi-idaei*. These fungi cause massive infections of *Prunus domestica* and *Rubus idaeus* and release large amounts of small-sized spores into the air. Additionally, their distribution depends largely on climatic conditions and human activity. For the first time, we present the cytotoxic potential of spores of the above-mentioned fungi against normal human bronchial epithelial cells.

Aim: To evaluate the cytotoxic activity of crude fungal extracts of *Tranzschelia pruni-spinosae* and *Phragmidium rubi-idaei* against normal human bronchial epithelial cells.

Methods: Normal human bronchial epithelial cells (BEAS-2B cell line) were used in this study. The sensitivity of the cells to both fungal extracts applied in a concentration range of 400-0.09 µg of protein/ml was determined by the MTT assay. To confirm the MTT assay results, cytometric analyses with annexin and propidium iodide were performed to determine live, apoptotic, and necrotic cells. Dihydrorhodamine 123 was used to test production of reactive oxygen species (ROS) as the possible cause of cell death.

Results: Both extracts showed dose-dependent cytotoxic activity – a reduction in the percentage of live cells by about 20-50% was noted in the concentration range of 400-100 µg of protein/ml. These changes appeared simultaneously with a significant increase in the percentage of apoptotic and/or necrotic cells. The percentage of bronchial epithelial cells producing ROS increased only after exposure to the *Tranzschelia pruni-spinosae* extract at the concentration of 400 and 100 µg of protein/ml.

Conclusion: *Tranzschelia pruni-spinosae* and *Phragmidium rubi-idaei* exhibit cytotoxic activity against normal human bronchial epithelial cells, which suggests their pro-inflammatory potential.

The research was financed by the NCN OPUS 2019/35/B/NZ6/00472 project.

5. Autoimmunity and Autoinflammation

The molecular basis of autoimmune diseases

Natalia Wawrusiewicz-Kurylonek[1,2]

1 – Department of Clinical Genetics, Medical University of Bialystok, Bialystok, Poland
2 – Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Bialystok, Bialystok, Poland

Autoimmune diseases (ADs) have always been a serious challenge for modern medicine, especially due to their increasing incidence and chronic character. The aetiology of autoimmune diseases is still unknown, but it is suggested that they are mainly influenced by both genetic factors and many environmental and complex interactions between them can influence of the development of autoimmunity. The knowledge of the pathomechanisms of autoimmune diseases has been growing rapidly over the last decade. Interactions of a number of environmental and molecular-genetic factors may contribute to the induction, development and progression of autoimmune diseases. A lot of numerous data indicate that environmental factors acting on a genetically sensitive organism can directly initiate, modulate or intensify the dysregulation of immune homeostasis and the development of a pathological process of autoimmunity. They can also stimulate the formation of mutations/ polymorphic variants in genes that encode the factors that regulate the functioning of the immune system, leading to modification of immune tolerance, regulatory pathways and immunosuppression processes. The existence of genetic background indicates coexistence of ADs among families and compatibility indicators in dizygous and monozygous twins. The genome-wide association studies carried out successfully in the last decade based on a single nucleotide polymorphisms tests in high-volume cohorts provide information on new 'candidate genes' exhibiting association with multiple ADs.

Due to the complexity of mechanisms inducing the process of autoimmunity in the course of ADs, the involvement of many genetic and environmental factors that increase the risk of their development, as well as a lack of collective knowledge on pathogenesis, it is reasonable to know and understand precisely the potential role of the a lot of genetic variants. The analysis of co-occurrence of a specific risk or protection variants in particular groups of patients with assessed biochemical parameters help to deepen the knowledge on the pathogenesis of ADs and may also help to develop more effective methods to control the loss of immune balance of homeostasis in patients. Knowledge of the individual components involved in the pathogenesis of these diseases may in the near future be the basis for the development of optimal preventive measures, enabling early diagnosis or even a choice of effective and efficient and innovative treatment.

Autoinflammatory diseases in adults: own experience in treatment with interleukin

Ewa Więsik-Szewczyk

Department of Internal Medicine Pulmonology Alergology and Clinical Immunology, Military Institut of Medicine, Warsaw

Systemic autoinflammatory diseases (SAIDs) were first defined as a separate and new group of diseases only 20 years ago. Inflammasomes which are a type of intracellular multiprotein platform that triggers the production of IL-1β plays a predominant pathogenic role. This translates into clinical practice since biologics that block IL-1 activity are effective treatment option. By 2017, Polish patients did not have access to reimbursed treatment with IL-1 blockers. The breakthrough came in October 2017 with the launch of the Congenital Autoinflammatory Syndromes Treatment Programme, reimbursed by the Ministry of Since then anakinra, short acting Il-1 blocker is available for polish patients. Currently 34 adults were assessed to receive anakinra: specifically with CAPS, TRAPS, Schnitzler Syndrome and other syndromes related to IL 1 overactivity. In the presentation the aspects of SAIDs pathogenesis and the aspects of treatment efficacy and safety will be given.

Treatment program for congenital autoinflammatory syndromes – two years of pediatric experience

Beata Wolska-Kuśnierz

Immunology Department, Children's Memorial Health Institute

Systemic autoinflammatory diseases (SAIDs) were first defined as a separate and new group of diseases only 20 years ago. Inflammasomes which are a type of intracellular multiprotein platform that triggers the production of IL-1β plays a predominant pathogenic role. This translates into clinical practice since biologics that block IL-1 activity are effective treatment option. By 2017, Polish patients did not have access to reimbursed treatment with IL-1 blockers. The breakthrough came in October 2017 with the launch of the Congenital Autoinflammatory Syndromes Treatment Programme, reimbursed by the Ministry of Health and coordinated by the Autoinflammatory Diseases Section of the Rare Diseases Team. Since then anakinra, short acting Il-1 blocker is available for polish patients. Currently (Feb 2021), 25 pediatric patients were reported to receive anakinra. We present the experience to date in the qualification of patients for treatment with interleukin 1 blockers, evaluation of treatment results during the drug program. We present challenges that may improve the diagnosis and therapy of patients with autoinflammatory diseases in Poland.

Pathogenesis of experimental autoimmune myocarditis – insight into antigen-specific and bystander CD4$^+$ T cell responses

Przemysław Błyszczuk

Department of Clinical Immunology, Jagiellonian University Medical College, Cracow, Poland

Myocarditis is defined as inflammation of the heart muscle and has been acknowledged as in important cause of sudden death. In humans, infections with cardiotropic viruses or parasites often trigger heart-specific autoimmunity. Autoimmune responses might result in ongoing inflammation and heart tissue remodelling, which finally leads to dilated cardiomyopathy (DCM) – a pathogenic condition characterized by fibrotic changes in the myocardium, ventricular dilation and systolic dysfunction.

Experimental autoimmune myocarditis (EAM) represents a non-infectious animal model of myocarditis and DCM reflecting key aspects of the human disease. In the EAM model, susceptible mice are immunized with α-myosin heavy chain alpha (α-MyHC) in the presence of complete Freund's adjuvant. Immunized mice develop CD4$^+$ T cell-dependent myocarditis, characterized by an extensive infiltration of cardiac tissue with inflammatory cells mainly of the myeloid lineage. In EAM, inflammation is replaced by progressive cardiac fibrosis, ventricular dilatation and systolic dysfunction.

Using the EAM model we confirmed that heart infiltrating CD4$^+$ T cells expressed exclusively effector (T_{eff}) phenotype. By using adoptive transfer experiments, we showed that while heart-specific T_{eff} infiltrated the heart shortly after injection, heart non-specific T_{eff} effectively accumulated during myocarditis and became the major heart-infiltrating CD4$^+$ T cell subset at later stage. Restimulation of co-cultured heart-specific and heart non-specific CD4$^+$ T cells with α-MyHC antigen showed mainly Th1/Th17 response for heart-specific T_{eff} and up-regulation of a distinct set of extracellular signalling molecules in heart non-specific T_{eff}. Adoptive transfer of heart non-specific T_{eff} in mice with myocarditis did not affect inflammation severity at the peak of disease but protected the heart from adverse post-inflammatory fibrotic remodelling and cardiac dysfunction at later stages of disease. Thus, our results lead to hypothesis that heart-specific T_{eff} aggravate cardiac inflammation, whereas heart non-specific T_{eff} cells effectively contribute to myocarditis and protect the heart from the dilated cardiomyopathy.

Proinsulin-specific T regulatory cells as possible adoptive therapy in type 1 diabetes

Mateusz Gliwiński[1], Dorota Iwaszkiewicz-Grześ[1], Anna Wołoszyn-Durkiewicz[2], Monika Tarnowska[1], Magdalena Żalińska[2], Matylda Hennig[2], Hanna Zielińska[1], Anna Dukat-Mazurek[1], Joanna Zielkowska-Dębska[1], Maciej Zieliński[1], Anna Jaźwińska-Curyłło[3], Radosław Owczuk[4], Przemysława Jarosz-Chobot[5], Artur Bossowski[6], Agnieszka Szadkowska[7], Wojciech Młynarski[8], Natalia Marek-Trzonkowska[9,10,11], Grażyna Moszkowska[1], Janusz Siebert[9], Małgorzata Myśliwiec[2,11], Piotr Trzonkowski[1,11]

1 – Department of Medical Immunology, Medical University of Gdańsk, Gdańsk, Poland
2 – Department of Pediatric Diabetology and Endocrinology, Medical University of Gdańsk, Gdańsk, Poland
3 – Regional Center of Blood Donation and Treatment, Gdańsk, Poland
4 – Department of Anaesthesiology and Critical Care, Medical University of Gdańsk, Gdańsk, Poland
5 – Department of Children's Diabetology, Medical University of Silesia, Katowice, Poland
6 – Department of Peadiatrics, Endocrinology, Diabetology with Cardiology Division, Medical University of Białystok, Białystok, Poland
7 – Department of Paediatrics, Diabetology, Endocrinology and Nephrology, Medical University of Łódź, Łódź, Poland
8 – Department of Paediatrics, Oncology, and Haematology, Medical University of Łódź, Łódź, Poland
9 – Department of Family Medicine, Laboratory of Immunoregulation and Cellular Therapies, Medical University of Gdańsk, Gdańsk, Poland
10 – International Centre for Cancer Vaccine Science, University of Gdańsk, Gdańsk, Poland Poltreg S.A, Gdańsk, Poland

Objective: In type 1 diabetes mellitus (T1DM), the β cells of the islet pancreas are destroyed by autoaggressive T cells not properly controlled by T regulatory cells (Tregs). We focused on possible mechanisms regulating the progression of this disease.

Methods: In the studies, patients were divided into three groups: patients with newly diagnosed T1DM (NDT1DM), patients with long-duration T1DM (LDT1DM) and patients with LDT1DM treated previously with polyclonal Tregs. It was also noted if the differences might be dependent on the antigen specificity of Tregs expanded for clinical use and autologous sentinel Tconvs.

Results: In group of patients with LDT1DM we noticed T-cell immunosenescence-like changes and expansion of similar vβ/T-cell receptor (TCR) clones in Tconvs and Tregs. The treatment with Tregs was associated with some inhibition of these effects. Patients with LDT1DM were characterized by increased percentage of various proinsulin-specific T cells but not GAD65-specific ones. The percentages of all antigen-specific subsets were higher in the expansion cultures than in the peripheral blood. The proliferation was more intense in proinsulin-specific Tconvs than in specific Tregs but the levels of some proinsulin-specific Tregs were exceptionally high at baseline and remained higher in the expanded clinical product than the levels of respective Tconvs in sentinel cultures.

Conclusions: T1DM is associated with immunosenescence-like changes and reduced diversity of T-cell clones. Preferential expansion of the same TCR families in both Tconvs and Tregs suggests a common

trigger/autoantigen responsible. Interestingly, the therapy with polyclonal Tregs was associated with some inhibition of these effects. Proinsulin-specific Tregs appeared to be dominant in the immune responses in patients with T1DM and probably associated with better control over respective autoimmune Tconvs.

Trial registration number EudraCT 2014-004319-35.

This work was supported by the National Centre for Research and Development, Poland (grant numbers LIDER/160/L-6/14/NCBR/2015 and STRATEGMED1/233368/1/NCBR/2014).

Autologous extracellular Hsp70 exerts a dual role in rheumatoid arthritis

Stefan Tukaj[1], Jagoda Mantej[1], Michał Sobala[2], Katarzyna Potrykus[2], Krzysztof Sitko[1]

1 – Department of Molecular Biology, Faculty of Biology, University of Gdańsk, Wita Stwosza 59, 80-308, Gdańsk, Poland
2 – Department of Bacterial Molecular Genetics, Faculty of Biology, University of Gdańsk, Wita Stwosza 59, 80-308, Gdańsk, Poland

Extracellular heat shock proteins (Hsp) influence the adaptive immune response and may ameliorate pathogenesis of autoimmune diseases. While some preclinical observations suggest that highly conserved bacterial and/or murine Hsp70 peptides have potential utility in treatment of rheumatoid arthritis (RA) via induction of T regulatory cells (Treg), the role of extracellular inducible human Hsp70 in adaptive immune processes requires further investigation. The present study evaluated Hsp70 influence on inflammatory cytokine-mediated modulation of T cell immunophenotype in ways that influence RA onset and severity. Initial experiments in the present investigation revealed that serum levels of Hsp70 are approximately 2-fold higher in RA patients versus healthy control subjects. To explore the effect of extracellular Hsp70 on key processes underlying the adaptive immune system, the effects of a highly pure, substrate-, and endotoxin-free human Hsp70 on polarization of the T helper cell subpopulations, including $CD4^+IL\text{-}17^+$ (Th17), $CD4^+FoxP3^+$ (Treg), $CD4^+IFN\text{-}\gamma^+$ (Th1), and $CD4^+IL\text{-}4^+$ (Th2), were studied in naïve human peripheral blood mononuclear cell (PBMC) cultures stimulated with anti-CD3/28 mAb. Major findings included an observation that while Hsp70 treatment increased Th17 frequencies and Th17/Treg ratio, the frequency of Th1 cells and the Th1/Th2 ratio were significantly decreased in the Hsp70-treated PBMC cultures. Moreover, data shown here provides preliminary suggestion that major contributing Hsp70-mediated immunomodulation includes interleukin 6 (IL-6) influence on Th17/Treg and Th1/Th2, since expression of this inflammatory cytokine is enhanced by in vitro Hsp70 treatment. These results are nevertheless preliminary and require further investigation to validate the above model.

Therapeutic implications of targeting heat shock protein 70 by immunization or antibodies in experimental skin inflammation

Stefan Tukaj[1], Jagoda Mantej[1], Michał Sobala[2], Katarzyna Potrykus[2], Zbigniew Tukaj[3], Detlef Zillikens[4], Ralf J. Ludwig[5], Katja Bieber[5], Michael Kasperkiewicz[6]

1 – Department of Molecular Biology, Faculty of Biology, University of Gdańsk, Gdańsk, Poland.
2 – Department of Bacterial Molecular Genetics, Faculty of Biology, University of Gdańsk, Gdańsk, Poland.
3 – Department of Plant Physiology and Biotechnology, Faculty of Biology, University of Gdańsk, Gdańsk, Poland.
4 – Department of Dermatology and Center for Research on Inflammation of the Skin, University of Lübeck, Lübeck, Germany.
5 – Lübeck Institute of Experimental Dermatology and Center for Research on Inflammation of the Skin, University of Lübeck, Lübeck, Germany.
6 – Department of Dermatology, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA.

Heat shock proteins (Hsp) are constitutive and stress-induced molecules which have been reported to impact innate and adaptive immune responses. Here, we evaluated the role of Hsp70 as a treatment target in the imiquimod-induced, psoriasis-like skin inflammation mouse model and related in vitro assays. We found that immunization of mice with Hsp70 resulted in decreased clinical and histological disease severity associated with expansion of T cells in favor of regulatory subtypes ($CD4^+FoxP3^+$/$CD4^+CD25^+$ cells). Similarly, anti-Hsp70 antibody treatment led to lowered disease activity associated with down-regulation of pro-inflammatory Th17 cells. A direct stimulating action of Hsp70 on regulatory T cells and its anti-proliferative effects on keratinocytes were confirmed in cell culture experiments. Our observations suggest that Hsp70 may be a promising therapeutic target in psoriasis and potentially other autoimmune dermatoses.

Comparison of gene expression profile of regulatory T cells in children with Selective IgA deficiency with or without autoimmune diseases/presence of autoantibodies.

Magdalena Rutkowska-Zapała[1], Agnieszka Grabowska[2], Anna Kluczewska[1], Anna Szaflarska[1], Marzena Lenart[1], Anna Pituch-Noworolska[1], Maciej Siedlar[1]

1 – Department of Clinical Immunology, Institute of Paediatrics, Faculty of Medicine, Jagiellonian University Medical College, Krakow, Poland
2 – Department of Medical Genetics, Institute of Paediatrics, Faculty of Medicine, Jagiellonian University Medical College, Krakow, Poland

Selective immunoglobulin A deficiency (IgAD) is the most common primary immunodeficiency in Caucasians, defined as decreased serum level of IgA in the presence of normal levels of other immunoglobulin isotypes. Because of the immunological dysregulation, approximately 20% to 30% of patients develop autoimmune disorders. However, clinical features of the autoimmune phenotype in these patients might be atypical and cause the delay of final diagnosis. Taking into account the involvement of regulatory T lymphocytes (Treg) in the pathogenesis of the development of autoimmunity, the aim of this study was to investigate the gene expression profile of Treg cells in SIgAD patient, with or without autoimmune diseases/presence of serum autoantibodies in comparison to healthy subjects.

Children with SIgAD as well as healthy control subjects, were selected from patients treated in our outpatient clinic of the Department of Clinical Immunology. All patients were diagnosed according to the criteria of the International Union of Immunologic Society. The study include children, between 5 and 14 years of age, retrospectively allocated into the one of 3 groups: Control (n=10), SIgAD with or without autoimmune diseases (n=5 and n=6, respectively). Absolute number of circulating Treg lymphocytes was determined using Human Regulatory T cell Staining Kit (eBiosciences). Treg cells were isolated from peripheral blood mononuclear cells using magnetic sorting. The analysis of transcriptome-wide gene- and exon-level expression profiles was done with microarray technology using Clariom D assays (Affymetrix) and Transcriptome Analysis Console (TAC) Software.

As a results, the highest level of circulating Treg cells was observed in the SIgAD patients in which presence of autoantibodies was confirmed. Moreover, micoarray analysis show the significant differences in gene expression profiles, as well as in alternative splicing, between analyzed groups. Gene expression profiles of Treg cells is currently being evaluated and should be updated in the poster.

6. Bacteriophages and Immunity

A study that went wrong- phage engineering and phage pharmacokinetics

Katarzyna Hodyra-Stefaniak, Karolina Lahutta, Joanna Majewska,
Zuzanna Kaźmierczak, Dorota Lecion, Marek Harhala, Weronika Kęska, Barbara Owczarek,
Ewa Jończyk-Matysiak, Anna Kłopot, Paulina Miernikiewicz, Dominika Kula,
Andrzej Górski, Krystyna Dąbrowska

Hirszfeld Institute of Immunology and Experimental Therapy, Wrocław, Poland

Bacteriophages draw scientific attention in medicine and biotechnology, including phage engineering, widely used to shape biological properties of bacteriophages. We developed engineered T4-derived bacteriophages presenting seven types of tissue-homing peptides. We evaluated phage accumulation in targeted tissues, spleen, liver and phage circulation in blood (in mice). Contrary to expectations, accumulation of engineered bacteriophages in targeted organs was not observed, but instead, three engineered phages achieved tissue titres up to 2 orders of magnitude lower than unmodified T4. This correlated with impaired survival of these phages in the circulation. Thus, engineering of T4 phage resulted in the short-circulating phage phenotype. We found that the complement system inactivated engineered phages significantly more strongly than unmodified T4, while no significant differences in phages' susceptibility to phagocytosis or immunogenicity were found. The short-circulating phage phenotype of the engineered phages suggests that natural phages, at least those propagating on commensal bacteria of animals and humans, are naturally optimized to escape rapid neutralization by the immune system. In this way, phages remain active for longer when inside mammalian bodies, thus increasing their chance of propagating on commensal bacteria. The effect of phage engineering on phage pharmacokinetics as mediated by the immune system should be considered in phage design for medical purposes.

Phage interactions with the immune system

Andrzej Górski[1,2], Krystyna Dąbrowska[1], Ryszard Międzybrodzki[1,2,3], Jan Borysowski[3], Ewa Jończyk-Matysiak[1], Marzanna Łusiak-Szelachowska[1], Beata Weber-Dąbrowska[1,2].

1 – Bacteriophage Laboratory, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences (HIIET PAS), Wrocław, Poland;
2 – Phage Therapy Unit, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences (HIIET PAS), Wrocław, Poland;
3 – Department of Clinical Immunology, Transplantation Institute, Medical University of Warsaw, 02-006 Warsaw, Poland.

According to our hypothesis phages present in the gut may migrate to blood, lymph and other tissues. This assumption has now been confirmed by other authors who have also pointed out that phages may transcytose human cells (especially epithelial cells). Furthermore, our studies have revealed how phages may interact with the immune system. Phages may induce humoral antibody production which depends on phage type, route and length of administration. Interestingly, the appearance of phage-neutralizing antibodies in patients' sera does not exclude good outcome of phage therapy. Phages may also modulate the functions of the immune system and those effects may be phage-specific and diverse. Some anti-inflammatory and immunoregulating effects of phages may exert beneficial effects in autoimmune reactions, allograft rejection and graft-versus-host reaction.

What is more, those effects can also be beneficial in treating COVID-19 – associated immune disturbances that could be lethal to patients.

Our studies thus emphasize that phage therapy may not only target specific bacteria but may also be part of the current drug-repurposing strategy offering novel means of therapy, especially in disorders with aberrant immunity including autoimmune diseases and allograft rejection. A specific phage could be optimally selected for use in PT from different phage strains recognizing a given bacterium, considering both its anti-bacterial activity and the type of immune response it may evoke. This is important in patients with immunodeficiencies, autoimmunity, allograft recipients, etc. who – pending the nature of their disease – may require immunostimulation or immunosuppression. Evidently, further research in this field may pave the way for phage repurposing in medicine.

Supported in part by funds from the National Science Centre, Poland for project No. 2018/31/B/NZ6/03999.

7. Current Advancements in Basic Immunology

Immunological aspects and function of non-coding microRNAs and extracellular vesicles

Krzysztof Bryniarski, Katarzyna Nazimek

Jagiellonian University Medical College, Department of Immunology, Krakow, Poland

Extracellular vesicles (EVs) are released by virtually all cells in the body and represent an important way of intercellular communication of immunological cells as well. Exosomes constitute EV-subpopulation of endosomal origin, characterized by 40-160 nm in diameter. They are involved in the transport of DNA, RNA, lipids and metabolites as well as cytosolic cell-surfaces. Recent studies suggest that EVs and exosomes play a significant role in regulating intercellular communication within the immune system.

Their presence in the pellet of ultracentrifuged biological samples and morphology is visualized by transmission electron microscopy, their size is commonly assessed by nanoparticle tracking analysis, the surface marker's expression is analyzed by cytometry and western blot and their specific density is estimated by buoyancy ultracentrifugation. EVs could be efficiently separated by the antigen-specific affinity chromatography, and their immune regulatory activity is intensively studied in murine models of contact and delayed-type hypersensitivities by treatment of actively immunized mice or adoptively transferred immune effector cells. Immune regulatory EVs, secreted by CD8+ suppressor T lymphocytes, induced through intravenous injection of a high dose of syngeneic erythrocytes into naive recipients, are surface-armed with hapten/antigen-specific IgM light chains delivered by B1 lymphocytes, activated by hapten/antigen skin immunization following tolerization with erythrocytes. These immune regulatory EVs carry miRNA-150 and, when injected via different routes to actively immunized mice, eliminate the antigen-specific effector cells of contact or delayed-type hypersensitivity reactions, which leads to prolonged immune tolerance to hapten or protein antigen. Interestingly, we reported oral route as the most efficient for EV's therapeutic administration. Our research findings also imply that, apart from natural sorting of miRNAs into EVs at the time of their biogenesis, extracellular RNA or synthetic miRNA may be loaded to EVs by passive adhesion or transfection, which expresses the great immune regulatory potential, enabling the desired effect on immune response, especially in compensating of dysregulated immune response.

The study was supported by Polish Ministry of Science and Higher Education (N41/DBS/000187).

Alveolar lymphocytes – facts and myths

Piotr Kopiński

Chair Gene Therapy, Collegium Medicum, Nicolaus Copernicus University, M. Skłodowskiej-Curie 9, 85-094 Bydgoszcz, Poland

Alveolar lymphocytes (AL) constitute a distinct population of lung lymphocytes, which is localized in pulmonary interstitium and can be evaluated by cytoimmunological analysis of bronchoalveolar lavage (BAL). AL demonstrate a phenotype and functional features of recently primed effector T cells; NK, B and NKT cells are uncommon. They play a key role in the specific antigen development, course and outcome of interstitial lung diseases (ILD). AL count and functions depend on their recruitment, potential activation, proliferation and programmed cell death (apoptosis).

There are some events suggesting AL in healthy people to be end-stage postmitotic lymphocytes. On the other hand, due to our observations, one percent of AL currently undergoes mitosis, about the same amount enters apoptosis. A range of normal reference values may be established for AL percentage in BAL, including their lower limit. Thus they occur in lungs as an immune physiological component, not contamination. Among AL there is a continuum of changes from healthy individuals, through subclinical alveolitis, up to patients with overt ILD. In ILD the number and also percentage of AL is increased mostly in granulomatous diseases (as sarcoidosis and exogenous allergic alveolitis).

T cells in alveoli are alternatively CD4+ T helper cells and CD8+ cytotoxic cells. However, both CD4+ and CD8+ carry similar markers of cytotoxicity, antigen presenting system, Th1/2/17 polarization as well as markers of susceptibility to apoptosis. CD4+ cells, considered their phenotype (e.g. granzymes expression), seem also to play a local role of cytotoxic cells. In this context it is not clear why AL in some clinical conditions are dominated by Th (as sarcoidosis, berylliosis, and methotrexate abuse) and some others by Tc (including smokers in which CD4/CD8 index is low, both in healthy subjects and in ILD).

Extracellular vesicles suppress immune response in an antigen-specific manner after coating with antigen-specific antibody light chains

Katarzyna Nazimek[1,2], Philip W. Askenase[2], Krzysztof Bryniarski[1,2]

1 – Department of Immunology, Jagiellonian University Medical College, ul. Czysta 18, 31-121 Krakow, Poland
2 – Section of Rheumatology, Allergy and Clinical Immunology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520-8013, USA

Background: Antibody light chains (LCs) were recently found to play an important role in activation of immune response. In contrast, very little is yet known on the putative down-regulatory activity of LCs. We observed that CD8+ suppressor T cells antigen-specifically inhibit contact hypersensitivity (CHS) reaction in mice by releasing extracellular vesicles (EVs). Our further research findings showed that these suppressor T cell-derived EVs can be coated with hapten-specific LCs both in vivo and in vitro. Therefore, the current studies investigated the actual role of hapten-specific LCs in EV-mediated suppression of CHS reaction.

Methods: Production of EVs by CD8+ suppressor T cells was induced by intravenous administration of a high dose of haptenated syngeneic erythrocytes in wild type mice or mice deficient in B cells, immunoglobulins or NKT cells. Then, EVs were coated with LCs of various specificities and the presence of LCs on EVs was analyzed cytometrically. The regulatory activity of LC-coated EVs was assessed in mouse CHS reaction.

Results: Suppressor T cell-derived EVs from tolerized mice of B-cell-deficient μMT-/-, NKT-cell-deficient Jα18-/- and immunoglobulin-deficient JH-/- strains were non-suppressive, unless coated with LCs of specificity strictly corresponding to the hapten used for sensitization and CHS elicitation in mice.

Conclusion: We found that B1-cell-derived LCs may coat EVs in vivo and in vitro to ensure the specificity of CHS suppression. This observation uncovers the down-regulatory function of antigen-specific LCs in EV-mediated immune suppression and implies an emerging role of LCs in directing the specificity of cell-to-cell communication. This, in turn, expresses great translational potential in designing nanovesicle carriers for specific targeting of desired cells.

The study was supported by Polish Ministry of Science and Higher Education by grant number K/ZDS/001429.

Genetic abnormalities within telomerase reverse transcriptase gene promoter region, telomerase activity and expression, and telomere length – comparison in oncological and haematological cell lines cultured *in vitro*

Marta Dratwa[1], Barbara Wysoczanska[1], Eliza Turlej[2], Artur Anisiewicz[2], Magdalena Maciejewska[2], Joanna Wietrzyk[2], Katarzyna Bogunia-Kubik[1]

1 – Laboratory of Clinical Immunogenetics and Pharmacogenetics, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland;
2 – Department of Experimental Oncology, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland;

Telomerase reverse transcriptase gene promoter (*TERTp*) constitutes a regulatory element capable to influence *TERT* expression (TE), telomerase activity (TA) and telomere length (TL). Three groups of cell lines were tested: oncological (A-172, A-431, NCI-H358, A-549, HT-29, Hs294T, UM-UC-3, KU-19-19, MDA-MB-231, MCF-7, LoVo), haematological (MV-4-11, KG1a, HL-60, K562, THP-1, KOPN-8, SEM, RCH-ACV, Z-138, MOLM-13, REC-1, MAVER-1, Rs4;11) and control cell lines (HaCaT, SC, MCF-10A). The presence of C228T and C250T mutations within the *TERTp* (ddPCR, Bio-Rad), *TERT* TE (TaqMan Gene Expression Assay, Applied Biosystems), TA and TL (qPCR assay kits ScienCell's) were analysed in *in vitro* cultured cells.

The C228T and/or C250T somatic mutations in *TERTp* were detected only in 5 solid tumour cell lines (A-172, A-431, Hs294T, UM-UC-3 and MDA-MB-231), but not in either of the haematological cell lines (p=0.0100). The C228T (–124 bp) mutation was detected in 4 cell lines: A-172, Hs294T, UM-UC-3, MDA-MB-231 (glioma, melanoma, bladder cancer and breast cancer) while one C250T (–146 bp) mutation was observed in the A-431 (epidermal skin cancer) cell line. Cell lines characterized by the presence of somatic mutations (MUT+) had shorter TL and lower TA compared to other oncological cell lines lacking these mutations. Moreover, in the MUT+ cell lines a linear correlation was observed between TE and TA (R=0.9708, p=0.0021). The TE in MUT+ cell lines were lower compared to cell lines without mutation (MUT-) and 3-fold higher compared to normal cell lines. Also, a linear correlation was observed between TL and TA in all cell lines (R=0.4431, p=0.0004), as well as in both subgroups: oncological (R=0.4875, *p*=0.0169) and haematological (R=0.4719, p=0.0095) cell lines. Analysis of relative TE showed that haematological cell lines exhibited 11-fold higher TE compared to solid tumour cell lines (p=0.0007) and their mean expression was also 31- fold higher compared to normal cell lines (p=0.0071). Differences were also observed when haematological cell lines were stratified into following groups: acute lymphoblastic leukaemia (ALL: KOPN-8, SEM, RCH-ACV, RS4;11), mantle cell lymphoma (MCL: Z-138, Rec-1, Maver-1), acute myeloid leukaemia (AML: KG1a-M0, THP-1-M4, MV-4-11-M5b, MOLM-13-M5a, HL-60-M2/M3). As compared to the normal cell lines, the mean TE was 115-times higher for ALL, 20 times higher for MCL, 5 times higher for CML, and 15 times higher for AML cell lines.

Our results based on the *in vitro* model suggest that onco- and leukemogenesis processes may differ with regard to their *TERT* gene regulation mechanisms.

This was supported by the TARGETTELO project No. STRATEGMED3/306853 from the National Centre for Research and Development, Warsaw, Poland.

In search of markers of liver steatosis/fibrosis in children with Wilson's disease – the importance of L-FABP and LSDP5

Bierła Joanna B.[1], Konopka Ewa[1], Jańczyk Wojciech[2], Wierzbicka-Rucińska Aldona[3], Więckowski Sebastian[2], Obrycki Łukasz[4], Sarnecki Jędrzej[5], Cukrowska Bożena[1], Socha Piotr[2]

1 – Department of Pathomorphology, Children's Memorial Health Institute, Warsaw, Poland
2 – Department of Gastroenterology, Hepatology, Nutritional Disorders and Paediatrics, Children's Memorial Health Institute, Warsaw, Poland
3 – Department of Biochemistry, Radioimmunology and Experimental Medicine, Children's Memorial Health Institute, Warsaw, Poland
4 – Department of Nephrology and Arterial Hypertension, Children's Memorial Health Institute, Warsaw, Poland.
5 – Department of Radiology, Children's Memorial Health Institute, Warsaw, Poland.

Wilson disease (WD) patients, despite well known defect of copper transporter ATP7B, shows a spectrum of liver pathology ranging from asymptomatic status to changes manifested by elevated liver enzymes, liver steatosis (S) to clinically apparent cirrhosis (fibrosis, F). *In vitro* studies suggest that increased endogenous expression fatty acid-binding protein 1 (L-FABP) and lipid droplet-associated protein 5 (LSDP5) play a critical role in modulation of lipogenic gene expression, restoring of lipid droplet formation, elevation of lipid content, and inhibition of HSC activation. Thus the aim of the study was to assess the usefulness of FABP1 and LSDP5 measurements in sera of WD patients in relation to liver S/F.

The serum samples were collected from WD patients (n=64) and were stratified (on basis liver transient elastography – FibroScan®) into three groups: WD0 (n=19) without S and F; WD1 (n=37) with S and without F; WD2 (n=8) without S with F. Sera of healthy (n=10) and obese children (n=10, BMI>25, with S and without F) were used as controls. All sera samples were examined with ELISA Kit: Human L-FABP (HycultBiotech); Human Perilipin 5 (PLIN5) (MyBioSource). For statistical analyses T-student was used and polyserial correlation between tested parameters in Stata Statistical Software were examined.

There were no statistically significant differences in L-FABP levels between healthy, obese, WD0, WD1 children. However, statistically significant higher level of L-FABP was found in WD2 in comparison with healthy, obese, WD 0,WD1 groups. Stata analysis suggest positive correlation between level of L-FABP and liver F in WD, but correlation between level of L-FABP and S tended to be negative. There were no significant differences in PLIN5 levels between healthy, obese, WD0, WD2. However, statistically significant differences in level of PLIN5 between WD1 and obese or WD0 group were found. The correlation between level of PLIN5 and S tended to be negative.

8. Immune tolerance – from bench to bedside

Tolerance-induction with autologous tolerogenic dendritic cells treated with VITamin D3 and loaded with myelin peptides in Multiple Sclerosis (Tolervit-MS)

Mansilla MJ[1,2], Presas-Rodríguez S[3,4], Navarro-Barriuso J[1,2], Teniente-Serra A[1,2], Quirant-Sánchez B[1,2], Lopez-Díaz de Cerio AL[5], Inogés S[5], Prósper F[5], Ramo-Tello C[3,4] Martinez-Cáceres EM[1,2]

1 – Immunology Division, Germans Trias i Pujol University Hospital and Research Institute, Campus Can Ruti (Badalona, ES);
2 – Department of Cellular Biology, Physiology and Immunology, Universitat Autònoma de Barcelona;
3 – Multiple Sclerosis Unit, Department of Neurosciences, Hospital Universitari Germans Trias i Pujol (Badalona, ES);
4 – Department of Medicine, Universitat Autònoma de Barcelona;
5 – Cell Therapy Center, Clínica Universidad de Navarra (Pamplona, ES).

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system. Current treatments reduce disease activity but do not decrease long-term disability and have relevant side effects. Thus, there is an unmet need for safer and effective treatments. Autologous therapy with tolerogenic dendritic cells (tolDC) is a promising strategy for the attenuation of pathogenic T cells in autoimmune diseases. Our group has developed an antigen-specific cell therapy based on autologous vitamin D3 (VitD3)-tolDC loaded with myelin peptides.

In vitro studies have demonstrated a potent immunoregulatory activity of vitD3-tolDC reducing lymphocyte proliferation and IFN-g production and increasing IL-10 levels, in co-culture experiments. Moreover, *in vivo* studies in the animal model of MS revealed a beneficial effect of VitD3-tolDC ameliorating the severity of the disease. Considering these pre-clinical results, as well as reported outcomes from previous clinical trials using DC, a clinical trial is ongoing.

Active MS patients were included in a dose-escalation best-of-five design: Cohort 1 (5x10^6 VitD3-tolDC), Cohort 2 (10x10^6), Cohort 3 (15x10^6). The inclusion of a fourth Cohort of patients under IFN-beta treatment receiving the selected dose of VitD3-tolDC is ongoing. The trial protocol was approved by the Spanish regulatory authorities (AEMPS) (Nº EudraCT: 2015-003541-26, available at ClinicalTrials.gov Identifier: NCT02903537, Tolervit-MS). Each patient will receive 6 administrations of tolDC (first 4 every 2 weeks and last 2 every 4 weeks). Clinical, MRI and immunological monitoring of patients will be performed for 24 months.

From bench to bedside – Clinical therapy with T regulatory cells

Maciej Zieliński[1,2], Magdalena Żalińska[3], Dorota Iwaszkiewicz-Grześ[1,2], Mateusz Gliwiński[1,2], Matylda Hennig[3], Anna Jaźwińska-Curyłło[4], Halla Kamińska[5], Justyna Sakowska[1,2], Anna Wołoszyn-Durkiewicz[3], Radosław Owczuk[6], Wojciech Młynarski[7], Przemysława Jarosz-Chobot[5], Artur Bossowski[8], Agnieszka Szadkowska[9], Janusz Siebert[10], Natalia Marek-Trzonkowska[2,10,11], Małgorzata Myśliwiec[2,3], Piotr Trzonkowski[1,2]

1 – Department of Medical Immunology, Medical University of Gdańsk, Debinki 7, 80-210 Poland
2 – Poltreg S.A., Trzy Lipy 3 Street, 80-172 Gdańsk, Poland
3 – Department of Pediatric Diabetology and Endocrinology, Medical University of Gdańsk, Debinki 7, 80-210 Poland
4 – Regional Center of Blood Donation and Treatment, Hoene-Wrońskiego 4 80-210 Gdańsk, Poland
5 – Department of Children's Diabetology, Medical University of Silesia, Medykow 16, 40-752 Katowice, Poland
6 – Department of Anesthesiology and Critical Care, Medical University of Gdańsk, Debinki 7, 80-210 Poland
7 – Department of Pediatrics, Oncology and Hematology, Medical University of Lodz, Sporna 36/50, 91-738 Lodz Poland
8 – Department of Padiatrics, Endocrinology, Diabetology with Cardiology Division, Medical University of Bialystok, Jana Kilińskiego 1, 15-089 Białystok Poland
9 – Department of Pediatrics, Diabetology, Endocrinology and Nephrology, Medical University of Lodz, Sporna 36/50, 91-738 Lodz Poland
10 – Department of Family Medicine, Laboratory of Immunoregulation and Cellular Therapies, Medical University of Gdańsk, Debinki 2, 80-210 Poland
11 – International Centre for Cancer Vaccine Science, University of Gdańsk, Wita Stwosza 63, 80-308 Gdańsk, Poland

Background: Monotherapy with autologous expanded $CD4^+CD25^{high}CD127^-$ T regulatory cells (Tregs) or rituximab has been documented to slow disease progression in recent-onset type 1 diabetes mellitus (T1DM) patients. Whether a combined therapy including both drugs would further benefit this patient population is unknown.

Methods: We conducted a clinical trial TregVAC2.0/ISRCTN37116985 to explore the efficacy and safety of the combined treatment with Tregs and rituximab in pediatric patients with recent-onset T1DM The analysis included also previous studies testing monotherapies with Tregs or rituximab as monotherapies [TregVac1.0/ISRCTN06128462 and TN-05/NCT00279305, respectively]. The patients were allocated to 4 groups (aged 5-18 years; N=115): Tregs alone (N=25), rituximab alone (N=34), Tregs+rituximab (N=12), and control (N=44). The key primary efficacy analyses were C-peptide levels (mixed meal tolerance test [MMTT]) and the proportion of patients in remission at 12 and 24 months.

Findings: At month 24, compared to the control, only the combined therapy remained superior in area under the curve of C-peptide MMTT (treatment ratio, 1.912; 90% CI, 1.112-3.287). Additionally, the proportion of patients in remission was significantly higher in the combined therapy group (54.5%) than in either the Tregs (20.0%, P=0.048) or rituximab (28.1%, P=0.016) monotherapy group. Although adverse events (AEs) occurred in most (79%) patients, the rituximab group had the highest frequency ($p<0.001$). No AEs led to withdrawal of the study intervention or death.

Interpretations: Over two years, combined therapy with Tregs and rituximab was consistently superior to either monotherapy in delaying T1DM progression in terms of serum C-peptide levels and the maintenance of remission.

Funding: National Centre for Research and Development, Poland (grant no STRATEGMED1/233368/1/NCBR/2014) and EU Horizon 2020 Framework Programme (grant no: 830559 – TREG – H2020-EIC-SMEInst-2018-2020/H2020-SMEInst-2018-2020-2).

Trial registration: EudraCT: 2014-004319-35

Keywords: diabetes type 1, children, T regulatory cells,

The kinetics of cytokine secretion by cells obtained from mixed chimeric mice

Baśkiewicz-Hałasa M.[1], Hałasa M.[2], Piecyk K.[1], Rogińska D.[1], Schmidt Ch.[1], Machaliński B.[1]

1 – Department of General Pathology, Pomeranian Medical University, Szczecin, Poland
2 – Department of Human Nutrition and Metabolomics, Pomeranian Medical University, Szczecin, Poland

Background: Mixed chimerism is one of the most promising methods for the creation of immunological tolerance. The phenomenon can be obtained by hematopoietic stem/progenitor cell transplantation into properly prepared recipient. Although many studies were performed in this regard for the last few years, precise influence of mixed chimerism on host organism has still not been fully explained. Previously, we explored the impact of different mixed chimerism induction protocols on secretion of cytokines by cells obtained from mixed chimeric mice in the 8th week of study. In the current experiment, using our most efficient induction protocol, we focused on kinetics of cytokine secretion by the analysis of cytokines in the 3rd, 8th, and 20th week of study.

Methods: To induce mixed chimerism, C57BL/6J (H-2K^{b}; I-E^{-}) mice were exposed to 3-Gy total body irradiation (Day -1). Subsequently, these mice were treated with CD8-blocking (Day -2) and CD40L-blocking (Day 0 and 4) antibodies, followed by transplantation with 20x10^{6} Balb/c (H-2K^{d}; I-E^{+}) bone marrow cells (Day 0). The effectiveness of applied mixed chimerism induction protocol was confirmed previously by alloreactive lymphocytes elimination and monitoring of allogeneic skin graft survival. For assessment of selected cytokine (IFN-γ, IL-2, IL-4, IL-6, IL-10, IL-17A, and TNF) production, a total of 2x10^{6} spleen, bone marrow, and peripheral blood cells were incubated in 0,5 mL of Iscove's medium for 48 hours. Next, supernatants were analysed with BD CBA Mouse Th1/Th2/Th17 Cytokine Kit by flow cytometry and total mRNAs were isolated for QRT-PCR analysis.

Results: We stated decreased secretion of IFN- γ and IL-2 by spleen, bone marrow and peripheral blood cells, especially in the 20th week of study, which was confirmed by RQ-PCR method on mRNA level.

Conclusion: The establishment of the safe mixed chimerism induction protocol is not possible without precise determination of its influence on host organism. Our study contributes to better understanding of this phenomenon influence on the recipient organism.

Key words: cytokines, hematopoietic stem/progenitor cells, mixed chimerism, mice.

Investigation of a self-tolerogenic potential of suppressor T cell-derived extracellular vesicles

Katarzyna Nazimek[1,2,3], Eugenio Bustos-Morán[2], Noelia Blas-Rus[2], Bernadeta Nowak[1], Philip W. Askenase[3], Francisco Sánchez-Madrid[2], Krzysztof Bryniarski[1,3]

1 – Department of Immunology, Jagiellonian University Medical College, ul. Czysta 18, 31-121 Krakow, Poland
2 – Department of Immunology, Hospital de la Princesa, Autonomous University of Madrid, Health Research Institute of Princesa Hospital (ISS-IP), Calle Diego de León 62, 28006 Madrid, Spain
3 – Section of Rheumatology, Allergy and Clinical Immunology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520-8013, USA

Background: Intravenous administration of haptenated syngeneic mouse red blood cells (sMRBC) into mice induces CD8+ suppressor T cells that inhibit hapten-specific contact hypersensitivity (CHS) reaction, by releasing miRNA-150-carrying extracellular vesicles (EVs). Immune tolerance may also be induced by administration of erythrocytes carrying self-antigens. However, it is unclear whether self-erythrocytes may induce self-tolerance by themselves. Thus, our current studies investigated the possible self-tolerogenic potential of EVs induced by sMRBC intravenously administered to naive mice in a high dose.

Methods: sMRBC-induced EVs were characterized by flow cytometry and electron microscopy. Their self-tolerogenic action was determined in a newly developed mouse model of delayed-type hypersensitivity (DTH) to sMRBC and further assessed by cytometric evaluation effector T cell activation and apoptosis. DTH to sMRBC was characterized with the use of positive and negative selection assays.

Results: DTH to sMRBC is mediated by CD4+ T cells and macrophages. Intravenous administration of sMRBC leads to generation of bilamellar, CD9+CD81+ EVs that suppress sMRBC-induced DTH reaction in a miRNA-150-dependent manner. In addition, these EVs significantly reduce effector T cell activation and enhance their apoptosis.

Conclusion: Current findings described newly discovered mechanism of self-tolerance induced by intravenous delivery of a high dose of sMRBC and mediated by EV-carried miRNA-150. This implies the concept of naturally-occurring immune tolerance that may presumably be activated in a response to overloading of the organism with altered self-antigens.

The study was supported by Polish Ministry of Science and Higher Education by grants number K/ZDS/001429 and N41/DBS/000187.

9. Immunity and Stem Cells

Characteristics of mesenchymal stem cells obtained from adipose tissue in patients with rheumatic diseases

Ewa Kuca-Warnawin[1], Krzysztof Bonek[2], Marzena Olesińska[3], Piotr Szczęsny[3], Piotr Głuszko[2] and Ewa Kontny[1]

1 – Department of Pathophysiology and Immunology, National Institute of Geriatrics, Rheumatology and Rehabilitation, Warsaw, Poland
2 – Department of Rheumatology, National Institute of Geriatrics, Rheumatology and Rehabilitation, Warsaw, Poland
3 – Clinic of Connective Tissue Diseases, National Institute of Geriatrics, Rheumatology and Rehabilitation, Warsaw, Poland

Mesenchymal stem/stromal cells (MSCs) have immunosuppressive and regenerative properties. Adipose tissue is an alternative source of MSCs, named adipose-derived mesenchymal stem cells (ASCs). Because the biology of ASCs in rheumatic diseases (RD) is poorly understood, we performed a basic characterization of RD/ASCs phenotype and their immunomodulatory properties. ASCs were isolated from abdominal adipose tissue of systemic lupus erythematosus (SLE), systemic sclerosis (SSc), ankylosing spondylitis (AS) patients and healthy donors (HD). RD/ASCs have reduced basal levels of CD90. Compared with HD/ASCs, RD/ASCs produced similar amounts of prostaglandin E2 (PGE2), IL-6, leukemia inhibiting factor (LIF), and TGF-β1, more IL-1Ra, soluble human leukocyte antigen G (sHLA-G) and tumor necrosis factor-inducible gene (TSG)-6, but less kynurenines and galectin-3. All tested ASCs significantly decreased the number of proliferating T-cells, the number of division/proliferating cell, and fold expansion (replication index, RI), and there was similar significant up-regulation of kynurenines and PGE_2 in ASCs-PBMCs co-cultures. In co-cultures with activated CD4+ T cells and PBMCs, HD/ASCs and RD/ASCs downregulated CD25 and HLA-DR, while upregulated CD69 molecules expression with comparable potency. Thus RD/ASCs retain normal capability to regulate expression of activation markers on allogeneic T cells. We found that both RD/ASCs and HD/ASCs are able to shift Th differentiation to a functional anti-inflammatory direction only in the presence of accessory cells, whereas their direct effect may be proinflammatory.

This research was funded by the National Science Centre, Poland, grants number 2016/21/B/NZ5/00500 and 2015/17/D/NZ5/02219.

Crosslinks between innate immunity and mobilization of hematopoietic stem cells

Mariusz Z Ratajczak

Department of Regenerative Medicine, Medical University of Warsaw, Poland

Pharmacological mobilization of hematopoietic stem progenitor cells (HSPCs) from bone marrow (BM) into peripheral blood (PB) is a means to obtain HSPCs for hematopoietic transplantation. We postulated that this process is a result of mobilizing agent-induced "sterile inflammation" in the BM microenvironment due to complement cascade (ComC) activation. The first step is release of ATP in a pannexin-1-dependent manner from BM cells, and secreted from cells ATP, as an extracellular signaling nucleotide, triggers activation of the ComC and initiates the mobilization process. This process is triggered by extracellular ATP in P2X7- and P2X4- purinergic receptors-dependent manner. To support this both P2X7-KO and P2X4-KO mice are poor mobilizers. Activation of both receptors subsequently leads to activation in HSPCs of intracellular innate immunity pattern recognition receptor – Nlrp3 inflammasome, and Nlrp3-KO mice are also as we reported poor mobilizers. To explain involvement of Nlrp3 inflammasome in mobilization process, it promotes release from the cells in caspase-1-dependent manner of several alarmines that activate ComC. On the other hand extracellular ATP after its release into the extracellular space, is processed by ectonucleotidases: CD39 converts ATP to AMP, and CD73 converts AMP to extracellular adenosine. We observed that CD73-deficient mice mobilize more HSPCs than do wild type mice due to a decrease in adenosine concentration in the extracellular space, indicating a negative role for extracellular adenosine in the mobilization process. This finding has been confirmed by injecting mice with adenosine along with pro-mobilizing agents. In sum, we demonstrate for the first time that purinergic signaling involving extracellular ATP and its metabolite extracellular adenosine regulate the mobilization of HSPCs in an opposite way. While ATP triggers and promotes this process, adenosine has an inhibitory effect. We conclude that sterile inflammation induced by pharmacological mobilization is a result of the activation of purinergic signaling that triggers innate immunity pathways in the BM microenvironment involving both BM-residing innate immunity cells and soluble innate immunity mediators. Thus, administration of extracellular ATP together with G-CSF or AMD3100 or inhibition of CD73 by small molecule antagonists may provide the basis for more efficient mobilization strategies to obtain more HSPCs for hematopoietic transplantations.

Immunosuppressive properties of mesenchymal stem cells – perspectives of therapeutic administration

Marcin Moniuszko

Department of Regenerative Medicine and Immune Regulation, Medical University of Bialystok, Poland
Department of Allergology and Internal Medicine, Medical University of Bialystok, Poland

Mesenchymal stem/stromal cells (MSCs) are multipotent stem cells capable of differentiating into various adult cell types. Moreover, MSCs exert anti-inflammatory actions directed at numerous immune cells. Therefore, therapies based on MSCs were widely tested in preclinical experiments and their putative therapeutic potential has been suggested in several inflammatory diseases including acute lung injury, asthma, COPD, diabetes, arthritis, myocardial infarction and many others. Here we present several mechanisms of stem cell-mediated immunomodulatory actions in experimental and clinical settings of selected pulmonary diseases including COVID-19. Finally, we present preliminary data on genomic effects of intranasal MSC administration in experimental model of eosinophilic and neutrophilic asthma.

Can mesenchymal stem cells affect wound healing and organ remodeling?

Andrzej Eljaszewicz

Department of Regenerative Medicine and Immune Regulation, Medical University of Bialystok

Wound healing is a physiological process occurring in the reaction to structural tissue damages. It involves different cellular subpopulations acting through soluble mediators, including cytokines, chemokines, growth factors, and metabolites. Wound healing consists of four subsequent and partially overlapping phases, namely hemostasis, inflammation, proliferation (re-epithelization), and remodeling (scar maturation). Disruption of healing mechanisms by prolonged inflammation, oxidative stress, genetic aberrations, dysregulated angiogenesis, and impaired cellular metabolism, among others, may lead to the development of chronic wounds or ulcers. Notably, hardly healing wounds, chronic wounds, and ulcers represent a significant therapeutic challenge. Therefore, there is a substantial need to develop novel therapeutic strategies allowing wound closure and improving the healing process. Recently, mesenchymal stem cells (MSC) were shown to possess a high therapeutic potential to improve healing processes. MSCs are multipotent stromal cells identified and isolated from various human tissues, including bone marrow, adipose tissue, Wharton's jelly, cord blood, and amniotic fluid. They are characterized by low immunogenicity, immune regulatory properties, high regenerative potential, and the ability to differentiate into mesodermal lineage cells. However, MSC functional properties depend on their stimulation by microenvironmental factors (local within the tissue) or represent a consequence of their in vitro processing. In fact, however, all to date described functional properties of MSCs might affect wound healing and organ remodeling directly (by cell-to-cell interactions) or indirectly (by the release of soluble factors).

Transcriptomic profiling of MSC-mediated changes in the eosinophilic and neutrophilic lung inflammation revelated different putative mechanisms of immunosuppression

Marlena Tynecka[1], Adrian Janucik[1], Magdalena Niemira[2], Aleksandra Starosz[1], Arkadiusz Zbikowski[3], Kamil Grubczak[1], Dawid Groth[1], Anna Szalkowska[2], Urszula Korotko[4], Malgorzata Lukasik[5], Joanna Reszec[5], Miroslaw Kwasniewski[4], Adam Kretowski[2,6], Marcin Moniuszko[1,7], Andrzej Eljaszewicz[1]

1 – Department of Regenerative Medicine and Immune Regulation, Medical University of Bialystok, Bialystok, Poland
2 – Clinical Research Centre, Medical University of Bialystok, Bialystok, Poland
3 – Department of Medical Biology, Medical University of Bialystok, Bialystok, Poland
4 – Centre for Bioinformatics and Data Analysis, Medical University of Bialystok, Bialystok, Poland
5 – Department of Medical Pathomorphology, Medical University of Bialystok, Bialystok, Poland
6 – Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Bialystok, Bialystok, Poland
7 – Clinical Department of Allergic and Internal Diseases, Medical University of Bialystok, Bialystok, Poland

Mesenchymal stem cells (MSCs) gained great scientific interest due to their immunosuppressive and regenerative/reparatory properties. Initially identified in the bone marrow are successfully replaced in research and clinical trials by more accessible sources originating from adipose tissue, Wharton's jelly, and cord blood. In fact, MSC-based therapy holds a promise for an array of degenerative and inflammatory diseases, including severe asthma. Although significant progress has been made in understanding MSC's therapeutic properties, immunomodulatory mechanisms remain not fully elucidated.

Therefore, we aimed to better understand the mechanisms of MSC-mediated regulation of eosinophilic and neutrophilic experimental asthma.

C57BL6 mice were challenged with 10 and 100μg/ml house dust mite extract (HDM) to induce eosinophilic and neutrophilic lung inflammation, respectively. Adipose tissue-derived-MSCs were administrated i.n. on days 6 and 13 of the experiment. Mice were sacrificed on day 15. Histochemical staining was performed to assess the inflammatory response and collagen deposition within the lungs. Transcriptomic profiling (RNAseq) of whole lung tissue was performed using the Illumina platform. The data were analyzed using "R", Ingenuity Pathway Analysis (IPA), and GraphPad Prism.

First, we confirmed the suppression of lung inflammation in both phenotypes after MSCs administration. Additionally, we observed reduced collagen deposition after MSC administration in lungs with neutrophilic inflammation. Next, the comparative analysis of differentially regulated genes among the analyzed groups was performed. Interestingly, the vast majority of differentially regulated genes after MSC administration were unique for both analyzed experimental asthma models. Consequently, distinct differentially regulated canonical and noncanonical pathways were defined in eosinophilic (dendritic cell maturation, IL-7, and B cell receptor signaling) and neutrophilic inflammation (lipid mediators' metabolism) upon MSCs administration. Finally, we observed intriguing changes in genes related to epithelial barrier integrity and airway remodeling after MSCs-administration.

In summary, by using a novel mice model of experimental asthma, we confirmed the therapeutic potential of adipose tissue-derived-MSCs. Importantly, we revealed novel distinct putative mechanisms of MSC-mediated immunosuppression in eosinophilic (immunologic) and neutrophilic (metabolic) lung inflammation.

The stimulatory effect of bone morphogenetic protein 2 (BMP-2) and fibroblast growth factor 2 (FGF-2) on osteogenic differentiation of sheep bone marrow-derived mesenchymal stem cells (BM-MSCs) *in vitro*

Sandra Gromolak[1], Agnieszka Krawczenko[1], Agnieszka Antończyk[2], Krzysztof Buczak[2], Zdzisław Kiełbowicz[2], Aleksandra Klimczak[1].

1 – Laboratory of Biology of Stem and Neoplastic Cells, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland;
2 – The Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, Wrocław, Poland

Background: Mesenchymal stem cells (MSCs) are of interests for bone tissue engineering due to their ability to osteogenic differentiation. Currently, approaches to stem cell-based bone regeneration based on utilization of growth factors, especially of BMP-2, are more effective for bone restoration. In this work, we studied the osteogenic potential of sheep bone marrow-MSCs (BM-MSCs) treated with BMP-2 and FGF-2.

Methods: MSCs were isolated from BM, aspirated from sheep iliac crest (n=12), and treated with 100 ng/ml BMP-2 and/or 20 ng/ml FGF-2. The effect of cytokines on proliferative activity was evaluated by MTT assay after 24, 48, 72 and 96 hours of incubation. Flow cytometry was used to analyze stem cell markers. The osteogenic differentiation potential was confirmed by Alizarin Red S staining and immunofluorescence detection of osteogenic-related proteins: collagen type I and osteocalcin. The effect of BMP-2 and FGF-2 on osteogenic stimulation of BM-MSCs was also investigated by qPCR for osteogenic lineage markers: BMP-2, Runx2, Osterix (Osx), Collagen I (ColI), Osteocalcin (Ocl) and Osteopontin (Opn).

Results: Cell proliferation of the BM-MSCs treated only with FGF-2 was higher than those treated with both BMP-2 and FGF-2. The expression of cell surface markers CD73, CD90 and CD105 was similar in both groups. There was a lack of expression of CD34, CD45 and MHC class II antigens. BMP-2 enhanced bone mineralization capacity of BM-MSCs as confirmed by Alizarin Red S staining, immunodetection of collagen I and osteocalcin, and upregulation of mRNA for early (BMP-2, Runx2, Osx) and late (ColI, Ocl, Opn) osteogenic differentiation genes.

Conclusion: Sheep BM-MSCs, with characteristic of MSC surface markers, are able to differentiate into osteogenic lineage. Culture medium supplemented with FGF-2 and BMP-2 improves their osteogenic differentiation capability. Therefore, preconditioning of BM-MSCs with these cytokines can be an effective strategy for cellular therapy in bone regeneration.

Transcriptomic profiling of mesenchymal stem cell (MSCs)-mediated effects on a healthy lung

Adrian Janucik[1], Marlena Tynecka[1], Magdalena Niemira[2], Arkadiusz Zbikowski[3], Aleksandra Starosz[1], Kamil Grubczak[1], Anna Szalkowska[2], Urszula Korotko[4], Malgorzata Lukasik[5], Joanna Reszec[5], Miroslaw Kwasniewski[4], Adam Kretowski[2,6], Marcin Moniuszko[1,7], Andrzej Eljaszewicz[1]

1 – Department of Regenerative Medicine and Immune Regulation, Medical University of Bialystok, Bialystok, Poland
2 – Clinical Research Centre, Medical University of Bialystok, Bialystok, Poland
3 – Department of Medical Biology, Medical University of Bialystok, Bialystok, Poland
4 – Centre for Bioinformatics and Data Analysis, Medical University of Bialystok, Bialystok, Poland
5 – Department of Medical Pathomorphology, Medical University of Bialystok, Bialystok, Poland
6 – Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Bialystok, Bialystok, Poland
7 – Department of Allergology and Internal Medicine, Medical University of Bialystok, Bialystok, Poland

Mesenchymal stem cells (MSCs) are spindle-shaped, plastic-adherent cells that possess high immunoregulatory properties. Due to their high therapeutic potential, MSCs gained scientific and clinical interest. However, due to the lack of sufficient data on the mechanisms of beneficial effects and their stability in vivo, their use in clinical practice is still minimal.

Here, we aimed to evaluate the effects of MSCs on the healthy normal lung using transcriptomic analysis.

MSCs were transferred i.n. to female C57BL/6 mice lung 2 (short-term) or 9 (long-term) days before the experiment termination. Hematoxylin & Eosin staining was performed to assess the lung inflammation. Next, whole lung mRNA was isolated and subjected to the Next Generation Sequencing (Illumina). "R" software and Ingenuity Pathways Analysis (IPA) software were used to analyze the obtained data. Genes from the differential gene expression analysis, acquired using the DESeq2 method, were subjected to the Gene Ontology term enrichment tool.

H&E staining showed a lack of inflammatory infiltrates within the lung in all analyzed samples. Interestingly, transcriptomic profiling of the lung tissue revealed 674 genes to be uniquely regulated in the short-term model, but only 76 in the long-term model, whereas 104 were shown to be commonly regulated. Moreover, IPA analysis revealed distinct differentially regulated canonical and non-canonical pathways among analyzed models. We found B cell receptor, IL-7, and HIF1α signaling to be the top 3 differentially regulated pathways in the short term-model. In some contrast long-term model was characterized by differential regulation of breast cancer regulation by Stathmin1, systemic lupus erythematosus in T cell signaling, and SAPK/JNK pathways. Furthermore, Gene Set Enrichment Analysis revealed both the sensory perception of smell and chemical stimulus biological processes to be the most significant terms enriched among those two models.

In summary, we showed that the administration of MSCs to the normal healthy lungs does not induce any adverse effects and thus confirmed the safety of MSCs usage. However, we found intriguing changes in the transcriptomic profiles of mice lungs. Further studies are needed to understand the temporary and long-lasting effects of MSCs administration on the lungs.

Microvesicles derived from stem/progenitor cells as carriers of bioactive factors facilitating angiogenesis

Agnieszka Krawczenko, Aleksandra Bielawska-Pohl, Maria Paprocka, Honorata Kraskiewicz, Agnieszka Szyposzynska, Elżbieta Wojdat, Aleksandra Klimczak

Laboratory of Biology of Stem and Neoplastic Cells, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, R. Weigla 12, 53-114 Wroclaw, Poland

Background: Endothelial progenitor cells (EPCs) and mesenchymal stem/stromal cells (MSCs) support maintaining of tissue homeostasis and tissue repair. Both types of cells contribute to tissue regeneration through the secretion of trophic factors, released also in the form of microvesicles (MVs). The isolation and biological properties of MVs derived from human immortalized MSC cell line HATMSC1 of adipose tissue origin and EPCs line were evaluated.

Methods: The human immortalized cell line derived from the adipose tissue of a patient with venous stasis was established using the hTERT and pSV402 plasmids. The human EPC line originating from cord blood (HEPC-CB.1) was established in our previous studies. Microvesicles were isolated using a sequential centrifugation protocol. Analysis of the protein content of both populations of MVs was performed using the Membrane-Based Antibody Array, whereas the expression of proangiogenic miRNAs was assessed by real-time RT-PCR with the TaqMan technique. The isolated MVs were assessed for their effect on the proliferation of cells involved in skin repair using cell lines: dermal endothelial cells (HSkMEC.2), fibroblasts (MSU-1.1), and keratinocytes (HaCaT), by standard MTT assay. Moreover, proangiogenic properties of isolated MVs on dermal endothelial cells were evaluated using tube formation assay in Matrigel.

Results: Analysis of the protein content of both populations of MVs revealed that isolated MVs transported growth factors (e.g., EGF, bFGF) and pro- and anti-angiogenic factors (e.g. IL-8, VEGF, TIMP-1, and TIMP-2). Additionally, EPC-derived MVs carried cytokines and molecules that regulate angiogenesis (e.g. GRO, IGF-I, I-TAC, MCP-1, MMP-1, VEGF-D). The presence of proangiogenic miRNA, i.e., miR-126, miR-296, miR-378, and miR-210 was documented in the isolated MVs. It was demonstrated that both HEPC-CB.1- and HATMSC1 derived MVs increased the proliferation of dermal endothelial cells (HSkMEC.2), and that this effect was dose dependent. In contrast, MVs had a limited impact on the proliferation of fibroblasts and keratinocytes. Both types of MVs improved the proangiogenic properties of human dermal endothelial cells, and this effect was also dose-dependent, as shown in the Matrigel assay.

Conclusion: These results confirm the hypothesis that MVs of HEPC-CB.1 and HATMSC1 origin carry proteins and miRNAs that support and facilitate angiogenic processes that are important for cutaneous tissue regeneration.

10. Immunity, aging and aging-related diseases

Proteodynamics and aging – relevance for aging-related diseases and the mechanisms of the immune system aging.

Jacek M. Witkowski

Department of Pathophysiology, Medical University of Gdańsk, Gdańsk, Poland

Although one cannot negate the roles of nucleic acids, lipids and sugars, main facets of cellular (and so, organismal) life depend and are executed by proteins. It is well established that proteins undergo many forms of posttranslational modifications, some necessary to render the protein functional (e.g., as an enzyme or structural protein), and some detrimental (like excessive aggregation and crosslinking). The latter processes are amplified during cellular and organismal aging, eventually leading to cellular dysfunction, senescence and death. Processes responsible for removal of aggregated, misfolded and otherwise dysfunctional proteins are commonly known as proteostasis. We went further, to stress that cellular aging depends not only upon failing proteostasis, but upon changes at each and every stage of protein formation, maturation and ultimately aging, and coined the term "proteodynamics" to reflect this. The immune system functionality depends on multiple protein systems, including those associated with cell proliferation (and fast production of immune cell precursors, as well as multiple forms of effectors and regulators of the immune response), involved in pathogen recognition (including pattern recognition and antigen receptors, and relevant signal transduction pathways), and in the actual immune reaction (including, but not limited to those involved in phagocytosis and intracellular killing of pathogens, antigen presentation, hundreds of cytokines, myriads of antibodies etc.). All of these undergo qualitative and quantitative changes associated with aging of the immune system. This in turn may facilitate the occurrence and increase the severity of aging-associated diseases, including the metabolic syndrome, type-2 diabetes, autoimmune diseases, malignancies, neurodegeneration, cardiovascular diseases and, notably, also COVID-19. This lecture will summarize our current understanding of these processes and their consequences.

Assessment of regulatory T cells senescence in *in vitro* culture

Justyna Sakowska[1], Maciej Zieliński[1], Piotr Trzonkowski[1]

1 – Department of Medical Immunology, Medical University of Gdańsk

Background: Regulatory T cells (Tregs) are one of the most important players in immune tolerance maintenance. Age related impairment of the immune system (immunosenescence) and replicative cell senescence defined as arrest of proliferative ability due to DNA damage and antigen shedding may have detrimental effect on Tregs suppressive abilities and cause clinical consequences. We are looking for the best fitting markers of Tregs senescence process, that can predict functionality of senescent cells and distinguish pathological aging process. Additionally, we look into pathways in control of cell aging that show characteristic constitutive activation of p38 MAP kinase in senescent cells.

Methods: To assess senescence severity of sorted Tregs during 12 days of *in vitro* culture we analyze a wide cellular phenotype corresponding to senescent cells and relative telomere length quantification, as well as activity of the enzyme telomerase. Secondly, levels of phosphorylation of proteins in p38 MAPK and mTOR kinases pathways are measured to investigate activation of cells in time. To asses Tregs at the functional level, their ability to suppress the immune response is tested by performing a suppression assay where Tregs are used to inhibit effector CD4 T cells stimulated by anti-CD3/CD28 antibodies.

Preliminary results: We observed that Tregs stimulated *in vitro* with anti-CD3/CD28 antibodies did not significantly shorten telomeres although the activity of telomerase is higher by the end of 12-days culture. In phenotype of Tregs a pronounced shift of cells toward memory compartment at day 12 compared to day '0' and slight decrease in expression of Foxp3 is observed during culture. Interestingly, phosphorylation of p38 MAP kinase and S6 protein of mTOR pathway is higher at day 7 and drops by the end of culture. Suppression assay shows similar suppression capabilities of Tregs at day 7 and day 12 of culture.

Conclusion: By day 12 of *in vitro* culture Tregs are highly activated, which is discernible as upregulation of telomerase and phosphorylation of kinases pathway proteins, and, most importantly, Tregs maintain their suppressive function. With further research we hope to distinguish markers that allow straightforward detection of senescent Treg cells and find the easiest way to identify viable, functional cells to use in cell therapy with Tregs.

Extracellular vesicles secreted by senescent vascular smooth muscle cells influence T cell functioning

Grażyna Mosieniak[1], Agata Ciolko[1], Dominik Cysewski[2], Dorota Janiszewska[1], Paulina Podszywałow-Bartnicka[3], Ewa Kozlowska[4], Ewa Sikora[1]

1 – Laboratory of Molecular Basis of Aging, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland;
2 – Mass Spectrometry Laboratory, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland;
3 – Laboratory of Cytometry, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland; [4]Department of Immunology, Institute of Zoology, Faculty of Biology University of Warsaw, Poland.

Cellular senescence is a stress response that leads to long-lasting proliferation cessation. The main trigger of senescence is unrepairable double strand DNA damage (DSB), resulting in activation of DNA damage response signaling pathway (DDR), which is responsible for cell cycle arrest. Senescent cells can be characterized by several morphological and biochemical changes that altogether make up senescence phenotype. One of the most prominent characteristics of senescent cells, called senescence associated secretory phenotype (SASP), is its ability of increased secretion of many bioactive factors like proinflammatory cytokines, chemokines, matrix modifying enzymes etc. Importantly, senescent cells accumulate during aging of the organism, but they can also actively participate in the development of age-related diseases, like atherosclerosis. Indeed, increased number of senescent vascular smooth muscle cells (VSMCs) has been identified in the atherosclerotic plaque. It was postulated that diminished proliferation ability of senescent VSMCs limit plaque stability, increasing the danger of plaque rapture. In this context also SASP factors remain an important element of atherosclerotic plaque microenvironment that could influence immune cells, which are important players in atherosclerosis development and progression. The aim of our study was to characterize the SASP phenotype of senescent VSMCs including extracellular vesicles secreted by those cells using proteomic analysis and to investigate the influence of senescent cells derived EVs on T cells functioning. We identified almost 1000 soluble factors and similar number of proteins present in extracellular vesicles fraction released by VSMCs. We were able to distinguish the proteins most abundantly secreted by senescent cells as well as those that were underrepresented in comparison to secretome of control cells. Moreover, using T lymphocytes isolated from the buffy coats of healthy volunteers we examined the influence of EVs secreted by senescent VSMCs on T cell activation as well as secretion of selected cytokines. Altogether our results indicate that SASP components derived from senescent VSMCs have immune modulatory effect, which potentially can influence atherosclerotic plaque development.

This work is supported by grants National Science Centre UMO 2014/15/B/NZ3/01150, UMO-2018/31/B/NZ3/02931

11. Immunodermatology

Degradation of transcripts in skin homeostasis and pathogenesis

Agata Lichawska, Weronika Szukała, Piotr Konieczny, Jolanta Jura

Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow

Gene expression is regulated at many levels. One of them is post-transcriptional regulation including, inter alia, the control of the half-life of RNA molecules. Regulation of the level of transcripts is important in both physiological and pathological processes. Among the proteins involved in the process of transcript degradation, an important group are proteins with the PIN domain that function as endonucleases. Their representative is Monocyte Chemotactic Protein-1-Induced Protein 1 (MCPIP1) – an important negative regulator of inflammatory processes. Our research has shown that the MCPIP1 protein is present in the epidermis. Keratinocyte-specific MCPIP1 knockout mice (MCPIP1^{EKO} mice) developed spontaneous skin pathology, as a result of immune cell influx into the dermis and elevated inflammatory signalling. In addition, IMQ skin treatment resulted in accelerated development of psoriasis-like skin symptoms. Histological analyses confirmed the psoriasis-specific pathology of the skin, which was characterized by epidermal skin thickening, hyperkeratosis, and the retention of nuclei within corneocytes (parakeratosis). Immunohistochemistry showed a reduction in keratinocytes expressing Krt10 with the simultaneous increased expression of Krt14 in suprabasal epidermal layers. Furthermore, Mcpip1^{EKO} mice are more prone to the development of DMBA / TPA induced skin tumorigenesis. In developing tumors, changes in the level of many transcripts encoding proteins involved in the regulation of epidermal proliferation and differentiation as well as inflammatory processes and angiogenesis have been observed. Our research has confirmed the important role of MCPIP1 in skin homeostasis and pathogenesis.

This study was funded by OPUS grant 2016/23/B/NZ3/00792 (to J.J.)

Contemporary view on the pathogenesis of psoriasis in the context of modern therapy

Adam Reich

Department of Dermatology, Institute of Medical Sciences, Medical College of Rzeszow University, Poland

Psoriasis is a chronic, inflammatory skin disease with profound negative effect on the patients' quality of life. The pathogenesis of psoriasis is still not fully elucidated, but the genetic background and immunological disturbances play a major role and psoriasis is currently regarded as a Th1/Th17/Th22-mediated condition. More importantly, recent discoveries have resulted in the development of novel efficacious treatment options, which brought new hope for the most severely ill patients. The novel therapies are based on the blocking various cytokines by monoclonal antibodies or fusion proteins. To date, the most important and effective treatment strategies targets tumor necrosis factor (TNF), interleukin 17 (IL-17) and interleukin 23 (IL-23). These cytokines are key elements in the inflammatory cascade observed in psoriasis. TNF activates the nuclear factor kappa B (NF-κB) signaling pathway, which promotes cell survival and proliferation, and exhibits antiapoptotic effects on lymphocytes and keratinocytes. IL-17 induces proliferation of keratinocytes and production of proinflammatory cytokines, e.g. IL-1β, IL-6 and TNF, and antimicrobial peptides, such as β-defensin and matrix metalloprotease. Furthermore, dysregulation of IL-23 production promotes autoinflammation. Besides these cytokines, current treatment strategies also consider blocking of intracellular Janus kinases and phosphodiesterases. During the lecture, the recent discoveries in relation to pathogenesis and treatment of psoriasis will be discussed.

What is new in itch pathogenesis?

Jacek Szepietowski

Department of Dermatology, Venereology and Allergology, Wroclaw Medical University, Wroclaw, Poland

Itch (also termed pruritus) is the most common symptom in dermatology which has been described in various sources and contexts for many centuries. Defined as an unpleasant sensation leading to the urge to scratch, this sensation is divided into acute and chronic based on its duration. Chronic pruritus may be associated with a number of cutaneous (psoriasis, atopic dermatitis, urticaria etc.) or systemic disorders (renal, hematologic, neurologic, hepatic etc.), as well as due to drugs, or even as psychogenic itch. There are several epidemiological studies on pruritus in the general population, while studies in specific groups (such as cutaneous or systemic disorders) are more prevalent. The pathogenesis of pruritus is complex and still not fully understood. Despite its associations with pain, itch is currently regarded as a separate sensation, which is transmitted from free nerve endings through a complex pathway leading to the central nervous system. Various neuromediators, cytokines and other substances are associated with the development of itch. The crucial pathogenetic aspects of chronic itch, such as the interactions between various pruritogens and their receptors, and the description of itch pathways with regard to peripheral nervous system and central nervous system processing in different regions, including neural sensitization, have recently been raised. The knowledge about itch is constantly growing, which will undeniably result in new aspects concerning its pathogenesis and treatment in the future.

The role of ANCA antibodies in the diagnosis of small vessel vasculitis

Agnieszka Owczarczyk-Saczonek

Department of Dermatology, Sexually Transmitted Diseases and Clinical Immunology
The University of Warmia and Mazury, Olsztyn, Poland

According to Chapel Hill Consensus Conference the anti-neutrophil cytoplasmic antibodies are characteristic for small vessel vasculitis, as serological markers. There are three different kinds of ANCA can be distinguished according to their fluorescence pattern: cytoplasmic (c)-ANCA, perinuclear (p)-ANCA and atypic ANCA with indefinite pattern.

C-ANCA are highly specific for granulomatosis with polyangitis even over 90% of the cases. They are characterized by diffuse granular staining of the cytoplasm. The main antigen which reacts with the patient's sera is proteinase 3.

The second type pANCA is associated with the other ANCA mediated vasculitis: microscopic polyangitis, eosinophilic granulomatosis with polyangiitis and many types of glomerulonephritis, ulcerative colitis and in other autoimmune diseases in about 75% of the cases. The main antigen which reacting with the patient's sera is not only myeloperoxidase (MPO), but also elastase, lactoferrin, lysozyme or cathepsin G. Immunofluorescence is so characteristic: diffusing pattern around the nuclear membrane of granulocytes.

Interestingly, the presence of c- and p- ANCA was also described in different kinds of non-vasculitic disorders, such as inflammatory bowel diseases and systemic lupus erythematosus with a controversial clinical relevance.

In clinical practice, these antibodies are detected with indirect immunofluorescence such as a screening test for ANCA. When a positive IIF result is identified, the target antigen is confirmed by an antigen-specific immunoassay, especially ELISA, so specific and sensitive.

Improvements in antigen- capture methods have resulted in better assay performance. Nowadays, the newest and really comfortable BIOCHIP is used for screening more frequently. However determination of ANCA by indirect immunofluorescence assays combined with an antigen-specific ELISA is considered to be the gold standard in ANCA diagnostics.

The lecture discusses the diagnostic difficulties and methods of ANCA differentiation.

An own case illustrating the importance and importance of ANCA diagnosis in patients was discussed.

Immunological aspects of hidradenitis suppurativa

Łukasz Matusiak

Klinika i Katedra Dermatologii, Wenerologii i Alergologii Uniwersytetu Medycznego we Wrocławiu

Hidradenitis suppurativa (HS) is a chronic, recurrent, inflammatory dermatosis characterized by an occurrence of nodules, abscesses, sinus tracks and scarring, involving the body areas rich in apocrine sweat glands. Its pathogenesis is multifactorial and still not fully understood, therefore, current systemic therapies still remain a serious challenge. Extensive, chronic inflammatory response, as well as the immune dysregulation, are involved in the underlying pathomechanism of the condition. Increased numbers of several proinflammatory cytokines have been reported both in the blood and in the skin of patients suffering from HS, including TNFa, IL-1□, IL-17 and IL-23. These cytokines activate the endothelium of local blood vessels and induce the expression of a broad range of chemokines such as CXCL8, CXCL11, CCL2 and CCL20 in keratinocytes and CXCL1 and CXCL6 in fibroblasts. Together, these signals boost further infiltration of immune cells into the tissue, including granulocytes, T cells, B cells and monocytes, resulting finally (via inflammasomes) in pyroptosis, a highly inflammatory form of cell death and subsequently in excessive pus and tissue scarring observed in HS.

12. Immunology in Personalized Medicine

Heterogeneity of cytokines in rheumatoid arthritis – the road to personalized medicine

Ewa Bryl

Department of Pathology and Experimental Rheumatology, Medical University of Gdańsk, Poland

Rheumatoid arthritis (RA) is an inflammatory rheumatic disease in which the entire immune system is involved. Different aberrations of immune system were observed first locally in the joints and later also in the systemic blood circulation and in the bone marrow. Based on current knowledge regarding immunity of RA it seems that patients with RA diagnosis show a broad spectrum of changes. The heterogeneity of immune system of the patients includes humoral factors like different autoantibodies presented in the serum and different concentrations of cytokines in the serum, but also the cellular part of the immune system. Cytokines are soluble mediators and regulate majority of immune system processes. There is a big scientific interest in measurements of cytokines concentrations in blood of RA patients. However, huge heterogeneity was discovered and it seems to be a problem for clinical usage. This huge heterogeneity is dependent on many factors including genetic, epigenetic, and environmental factors, like smoking, obesity and diet. The stage of disease, age of onset, disease activity, treatment and patients' response to it can also contribute to this heterogeneity. Multiple measurements of cytokine concentrations in the same patient could serve as biomarkers allowing to follow up the changes during the disease course. In the future, such personalized assessment of changing cytokine concentrations in every single patient will be the best approach.

Personalized medicine in atopic dermatitis- where are we?

Magdalena Trzeciak

Katedra i Klinika Dermatologii i Wenerologii i Alergologii GUMed

Atopic dermatitis (AD) is a chronic, relapsing, inflammatory skin disease. It requires the therapeutic strategy that allows control the disease with its exacerbations. AD is characterized by persistent itch and skin lesions with a morphology typical for eczema and the location depending on the patient's age. In 20–30% the disease is assessed as moderate and severe. AD negatively influences quality of life of the patients and their families and has negative social and economic impact. It affects 15–25% of children and lasts till adulthood in 5–20% of cases. The prevalence in adults is estimated at 3–7%. Atopic dermatitis may coexist with other atopic diseases, such as allergic rhinitis, asthma or food allergy and with non-atopic diseases, such as autoimmune, cardiovascular, neurological and psychiatric diseases. Is there a target in the complicated pathogenesis of AD, capture of which would inhibit the development of the disease and its complications?

The pathogenesis of atopic dermatitis is complex indeed involving the genetic, the immunological and the environmental factors, with the epidermal barrier defect and the skin microbial dysbiosis. Undoubtedly, AD is a heterogeneous disease with variety of phenotypes and endotypes. It can be viewed through the molecular profile (disturbances in the epidermal barrier and immunological disorders) or through the geographic / ethnic profile. This knowledge helps to design and hopefully in the near future recommend targeted and individualized therapy. Biomarkers (in serum and skin) that would correlate with the risk of development, the severity of the disease, and treatment answers are still sought. Defining biomarkers in specific clinical and ethnic phenotypes can assist in therapeutic selection and trial design.

It remains to be hoped that the perspective of personalized medicine will become the therapeutic routine of AD.

Different responses of malignant myeloid cells to BCL-2 inhibitors – implications for acute myeloid leukemia therapy

Anna Mlyczyńska, Weronika Ortmann, Małgorzata Opydo-Chanek

Laboratory of Experimental Hematology, Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow

Myeloid cells originate from multipotent hematopoietic stem cell in bone marrow. During myelopoiesis, several factors influence survival, proliferation and differentiation of myeloid cells in part by regulation of BCL-2 family proteins. Members of BCL-2 family function as critical nodes in complex regulatory networks to make ultimate life/death decisions. Although BCL-2 family proteins do not have a direct role in myeloid cell proliferation and differentiation programs, these proteins can either permit these programs to proceed or prevent them. Through such effects, the BCL-2 family maintains cellular homeostasis. An alteration in the expression of its members disturbs a proper balance between cell death and cell survival and contributes to various hematological malignancies, including acute myeloid leukemia (AML). Moreover, elevated expression of pro-survival BCL-2 family proteins can be associated with chemoresistance and poor clinical outcome, suggesting that these proteins may be an attractive therapeutic target. Regarding this, the aim of the study was to compare the effects of Bcl-2 inhibitors, named BH3 mimetics, on malignant myeloid cells. The Western blot technique was used to detect the basal expression of BCL-2, MCL-1 and BCL-XL in several acute myeloid leukemia cell lines that represent various subtypes of AML. Leukemia cells were treated with BCL-2 inhibitors characterized by a different selectivity for anti-apoptotic proteins, such as ABT-199 (BCL-2 inhibitor), S63845 (MCL-1 inhibitor) and obatoclax (pan-BCL-2 inhibitor). The cytotoxic effects of these agents were evaluated by cell viability (PrestoBlue) and apoptosis (annexin V/propidium iodide) assays. As shown by the obtained results, BH3-mimetics induced a concentration-dependent decrease in cell viability and increase in apoptosis of AML cells. The most pronounced effects were seen after obatoclax treatment, however, both ABT-199 and S63845 also induced significant response, albeit at higher concentration than obatoclax. Western blot analysis revealed that the expression of BCL-2 family proteins was highly variable in AML cell lines what partially correlated with their sensitivity to BH3-mimetics. Overall, this data suggests that anti-apoptotic proteins of BCL-2 family are important therapeutic targets and the knowledge of Bcl-2 protein profile in AML cells is therapeutically relevant.

The study was supported by the grant from Polpharma Scientific Foundation.

13. Immunology of COVID-19

SARS-CoV-2 receptors in health and disease

Radzikowska U.[1,2,3*], Ding M.[1,2,4*], Tan G.[1,2,5], Zhakparov D.[1], Peng. Y.[1,2,6], Wawrzyniak P.[1,2,7], Wang M.[1,2,8], Li S.[1,9], Morita H.[1,2,10], Altunbulakli C.[1,2], Rieger M.[11], Neumann AU.[11,12], Lunjani N.[1,2], Traidl-Hoffman C.[2,11], Nadeau K.[13], O'Mahony L.[1,14], Akdis C.A.[1,2], Sokolowska M.[1,2]

*equal contribution

1 – Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, Davos, Switzerland
2 – Christine Kühne – Center for Research and Education (CK-CARE), Davos, Switzerland
3 – Department of Regenerative Medicine and Immune Regulation, Medical University of Bialystok, Bialystok, Poland
4 – Department of Allergology, Zhongnan Hospital of Wuhan University, Wuhan, China
5 – Functional Genomic Centre Zurich, ETH Zurich/University of Zurich, Zurich, Switzerland
6 – Otorhinolaryngology Hospital, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China
7 – Division of Clinical Chemistry and Biochemistry, University Children`s Hospital Zurich, Zurich, Switzerland; Children`s Research Center, University Children`s Hospital Zurich, Zurich, Switzerland
8 – Department of Otolaryngology, Head and Neck Surgery, Beijing TongRen Hospital, Capital Medical University and the Beijing Key Laboratory of Nasal Diseases, Beijing Institute of Otolaryngology, Beijing, China
9 – Department of Cancer Immunology, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway
10 – Department of Allergy and Clinical Immunology, National Research Institute for Child Health and Development, Tokyo, Japan
11 – Chair and Institute of Environmental Medicine, UNIKA-T, Technical University of Munich and Helmholtz Zentrum Munchen, Augsburg, Germany
12 – Institute of Computional Biology (ICB), Helmholtz Zentrum Munchen, Munich, Germany and Institute of Experimental Medicine (IEM), Czech Academy of Sciences, Prague, Czech Republic
13 – Sean N Parker Centre for Allergy and Asthma Research at Stanford University, Department of Medicine, Stanford University School of Medicine, Stanford, Calif
14 – Department of Medicine and School of Microbiology, APC Microbiome Ireland, National University of Ireland, Cork, Ireland

Background: Morbidity and mortality from COVID-19 caused by novel coronavirus SARS-CoV-2 is accelerating worldwide, and novel clinical presentations of COVID-19 are often reported. The range of human cells and tissues targeted by SARS-CoV-2, its potential receptors and associated regulating factors are still largely unknown. The aim of our study was to analyze the expression of known and potential SARS-CoV-2 receptors and related molecules in the extensive collection of primary human cells and tissues from healthy subjects of different age and from patients with risk factors and known comorbidities of COVID-19.

Methods: We performed RNA sequencing and explored available RNA-Seq databases to study gene expression and co-expression of ACE2, CD147 (*BSG*), and CD26 (*DPP4*) and their direct and indirect molecular partners in primary human bronchial epithelial cells, bronchial and skin biopsies, bronchoalveolar lavage fluid, whole blood, peripheral blood mononuclear cells (PBMCs), monocytes, neutrophils, DCs, NK

cells, ILC1, ILC2, ILC3, CD4+ and CD8+ T cells, B cells, and plasmablasts. We analyzed the material from healthy children and adults, and from adults in relation to their disease or COVID-19 risk factor status.

Results: *ACE2* and *TMPRSS2* were coexpressed at the epithelial sites of the lung and skin, whereas CD147 (*BSG*), cyclophilins (*PPIA* and *PPIB*), CD26 (*DPP4*), and related molecules were expressed in both epithelium and in immune cells. We also observed a distinct age-related expression profile of these genes in the PBMCs and T cells from healthy children and adults. Asthma, COPD, hypertension, smoking, obesity, and male gender status generally led to the higher expression of ACE2- and CD147-related genes in the bronchial biopsy, BAL, or blood. Additionally, CD147-related genes correlated positively with age and BMI. Interestingly, we also observed higher expression of CD147-related genes in the lesional skin of patients with atopic dermatitis.

Conclusions: Our data suggest different receptor repertoire potentially involved in the SARS-CoV-2 infection at the epithelial barriers and in the immune cells. Altered expression of these receptors related to age, gender, obesity and smoking, as well as with the disease status, might contribute to COVID-19 morbidity and severity patterns.

COVID-19: virus contra the immune system

Janusz Marcinkiewicz

Department of Immunology, Jagiellonian University Medical College, Krakow, Poland

Current opinions on the immune response to SARS-CoV-2 infection
The presentation will summarize the current knowledge of the immune response to SARS-CoV-2 infection in the COVID-19 pandemic. The following problems will be discussed:

- COVID-19 transmission – Airways droplet infection
- Clinical stages of COVID-19
- Lifecycle of SARS-CoV-2 in infected cells
- Defense mechanisms of mucosa in viral infection
- Innate and adaptive immunity in viral infections
- Course of COVID-19: Viral response phase *vs* hyperinflammatory phase
- Severe COVID-19: Cytokine storm – lung injury – ARDS – multiorgan failure
- High risks of severe COVID-19 outcome
- COVID-19 and the elderly patients: Immunosenescence – Inflammaging
- Antibody and T cell response to SARS-CoV-2 infection and vaccination
- Herd immunity against COVID-19, a hope to defeat the pandemic

The fate of COVID-19 patients is hanging in the balance between inflammatory and immunologic response to SARS-CoV-2

Andrzej Lange

Hirszfeld Institute of Immunology and Experimental Therapy, Wrocław

Summary of the talk.

The immune response to SARS CoV2 depends on:

- the pace of the adaptive immune response
- highly transmissible variants, shorten T cells repertoire, vascular bed damage are associated with the worst

Risk assessment

- assessing of the naive T cell pool, co-morbidity and the immunogenetic index should help in weaving out those at risk
- if the disease progresses enumeration of M-MDSCs may assist a decision-making process (steroids or immune response support)

mRNA Vaccine is effective but the immunologic signature of each variant may be essential for early recognition of the used vaccines potential

Vaccination against COVID-19, what are we expecting?

Joanna Zajkowska

Department of Infectious Diseases and Neuroinfections, Medical University of Białystok, Białystok, Poland.

The development of vaccines and the global immunization program is indisputably a success, but new challenges are emerging: not fast enough vaccination rate, the emergence of new variants and unequal access to vaccines. Vector and mRNA vaccines are a milestone in vaccinology. Do we need new vaccines or what we already have is enough? Pan-coronavirus vaccine is needed to protect against future outbreaks and pandemics. Is it possible? Hybrid, multi-variant vaccine seems to be an urgent need in the fight against new variants of the SARS COV-2 virus

Persistence of skewed T regulatory cells in patients recovering from severe COVID-19

Milena Wiech[1], Piotr Chroscicki[1], Marta Brewinska-Olchowik[1], Julian Swatler[1], Michal Hampel[2], Anna Maliszewska[2], Katarzyna Sklinda[3], Sara de Biasi[4], Marek Durlik[2,5], Waldemar Wierzba[6,7], Andrea Cossarizza[4], Katarzyna Piwocka[1]

1 – Laboratory of Cytometry, Nencki Institute of Experimental Biology, Polish Academy of Science, Warsaw, Poland
2 – Departament of Gastroenterological Surgery and Transplantology, Central Clinical Hospital of the Ministry of Interior, Warsaw, Poland
3 – Department of Radiology, Centre of Postgraduate Medical Education, Warsaw, Poland
4 – University of Modena and Reggio Emilia School of Medicine, Modena, Italy
5 – Departament of Gastroenterological Surgery and Transplantology, Centre of Postgraduate Medical Education, Warsaw, Poland
6 – Central Clinical Hospital of the Ministry of Interior, Warsaw, Poland
7 – University of Humanities and Economics, Lodz, Poland

Severe immune impairment, cytokine storm and inflammation are hallmarks of COVID-19. Dysfunctions in Foxp3+ T regulatory cells (Treg) also exist, related to the severe symptoms. However, it is still not clear whether, and how fast, the system recovers to the normal functionality, depending on the disease severity. Clinically, it is crucial to know whether patients recovering from COVID-19 should be considered immunologically healthy individuals. Here, we investigated whether the immune system of patients recovering from COVID-19 of different severity still display signs of uncontrolled inflammatory response and imbalanced Treg function.

We analysed 41 COVID-19-recovered male patients (median age 47, range 27-64), divided into three groups based on WHO classification of severity, as measured by lung lesions (confirmed by CT) and type of oxygen supply: severe (n=14), moderate (n=14) and mild (n=13), and 19 matching healthy controls. Blood samples were collected at 1-3 months after the negative PCR test and after another 3 months. Analyses were performed using 5-laser Cytek Aurora spectral cytometer. For functional measurement of cytokine production by T cells, PBMCs were stimulated with anti-CD3/CD28, treated with secretion inhibitor and followed by detection of CD3, CD4, CD8, and intracellular Foxp3, TGF-β, IL-17, TNF-α, IFN-γ, IL-2, granzyme B and CD107a. The plasma levels of 13 cytokines were quantified using a LEGENDplex™ HU Essential Immune Response Panel (BioLegend).

We observed a higher percentage of CD4+ cells that produced IL-2, TNF-α and CD107a in post-COVID patients recovering from severe disease. Also CD8+ subset from severe convalescent patients showed higher production of TNFα, IFN-γ and CD107a. Moreover, Treg from severe patients produced more IL-2, IL-2+IL-17, CD107a and TNF-α, in contrast to Treg from moderate and mild patients. Simultaneously, the cytokine levels in plasma show that the cytokine storm was already attenuated independently of the disease severity.

To sum up, patients who recovered from severe COVID-19, with >20% of lung lesions and active/mechanic oxygen supply still show dysfunctional and imbalanced immune system. Especially, the increased production of TNF-α, IFN-γ, IL-2, and IL-17 by CD4+ and Treg, indicates still existing promotion of the Th1/Th17 phenotype and skewed Treg activity. This might affect patients' health and should be considered before applying medical treatments to other disorders.

Funding: TEAM-TECH Core Facility Plus/2017–2/2 (POIR.04.04.00-00-23C2/17-00) grant from Foundation for Polish Science (KP).

Significance of NETs formation in COVID-19

Karolina Janiuk[1], Ewa Jabłońska[1], Marzena Garley[1]

1 – Department of Immunology, Medical University of Bialystok, J. Waszyngtona 15A, 15-269 Bialystok, Poland

Since the outbreak of acute infectious respiratory disease – COVID-19, caused by a novel strain of SARS-CoV-2 in late December 2019, healthcare systems around the world have had to face a serious challenge to their functioning. In a significant percentage of patients, COVID-19 leads to fatal respiratory failure associated with an excessive inflammatory response, caused by a cytokine storm. Understanding of the COVID-19 immunopathogenesis still remains incomplete, but emerging evidence emphasize the importance of increased infiltration and activity of neutrophils. To elucidate the key role of hyperactive recruited neutrophils in COVID-19 progression, many scientific reports highlight that this cells have the ability to form neutrophil extracellular traps (NETs). An excessive generation of NETs in the course of prolonged inflammation predisposes to the occurrence of a cascade of side effects, including thromboembolic complications and damage of surrounding tissues and organs. It is worth noting that NETs can activate neighboring macrophages to release pro-inflammatory cytokines, mainly IL-1β. The reverse situation was also observed, proving that IL-1β is a key inducer of NETs formation. This led to the hypothesis that there is a specific IL-1β-NETs feedback loop which supports the theory of cytokine storm severity. The aim of the presentation is to review prior reports linking exaggerated NETs formation to the higher mortality or severe course of COVID-19 in patients with underlying co-morbidities such as diabetes and cardiovascular diseases. Furthermore, the presentation summarize the current knowledge on the advances in drug research that have the ability to regulate NETs formation as important therapeutic strategies for COVID-19 treatment. Searching for as many strategic target points as possible for personalized therapies might be helpful in order to reduce the overall disease fatality rate of COVID-19.

Comparative analysis of three commercially available assays for quantitative measurement of anti-SARS-CoV-2 IgG in convalescent plasma

Agnieszka Żebrowska[1], Tomasz Wasiluk[1], Mateusz Dziemiańczuk[1,2], Marzena Smoktunowicz[1], Magdalena Bujno[1], Dariusz Średziński[1], Piotr Radziwon[1,2]

1 – Regional Centre for Transfusion Medicine, Bialystok, Poland
2 – Department of Hematology, Medical University of Bialystok, Bialystok, Poland

Introduction: One of the potential therapies of patients with COVID-19 is the transfusion of plasma from convalescents who have been infected with SARS-CoV-2 virus and have developed neutralizing antibodies (NAbs). Although the virus neutralization test remains the gold standard in determining antibody efficacy, due to its high cost, relatively low efficiency and restrictive microbiological safety requirements, alternative methods for determining the amount of anti-SARS-CoV-2 antibody in COVID-19 convalescent plasma (CCP) are used. Given the variety of antigen targets used in the SARS-CoV-2 antibody assays, it appears necessary to carefully evaluate these assays prior to widespread use in CCP donors.

Aim: Comparative analysis of three SARS-CoV-2 serological assays which measure the anti-SARS-CoV-2 IgG specific for antigen domains of the spike of the virus in convalescent plasma donors population. The results of the tested methods correlate with the titre of NAbs.

Materials and methods: In this case-controlled validation study, we used sera from 106 laboratory-confirmed COVID-19 cases (positive RNA-SARS-CoV-2 test). The control group consisted of 12 healthy plasma donors who had not been previously infected with SARS-CoV-2 virus. At the time of collection, the donors met the current convalescence criteria – two negative results of the SARS-CoV-2 RNA test and a period of at least 14 days from the repeat negative NAT test result. Each sample was tested with three research methods: Euroimmun SARS-CoV-2 IgG, Maglumi SARS-CoV-2 S-RBD IgG and Liaison SARS-CoV-2 S1/S2 IgG (Diasorin).

Results: Only CCP with high neutralising antibody titres should be used in the treatment of COVID-19 patients. The available data suggest that a titre of ≥1:160 might be an appropriate threshold to apply. Based on published evidence the corresponding titre of IgG comparable to the titre of NAbs is 1:500, which in our study correlate with S/Co ratio 4,4 (Anti-SARS-CoV-2 ELISA (IgG) EUROIMMUN), or 59,2 AU/ml (LIAISON SARS-CoV-2 S1/S2 IgG DiaSorin), or 27,4 AU/ml (MAGLUMI SARS-CoV-2 S-RBD IgG). Based on the comparative analysis, the positive results obtained with the Euroimmun, Diasorin and Maglumi tests were consistent. The further statistical analysis showed a positive linear correlation between the results. This interpretation is reinforced by the Spearman's rho correlation coefficiency, more resistant to outliers in the analysed sample, which also showed a positive correlation between the variables.

Conclusions: The analysed assays offer an optimal diagnostic accuracy for the quantitative determination of anti-SARS-CoV-2 IgG and may be acceptable tool for qualification of donated plasma for treatment of COVID-19 patients.

Key words: antibodies, COVID-19, ELISA, chemiluminescence, SARS-CoV-2, titers.

14. Immunology of Infection

Immune privilege of CNS – consequences for autoimmunity

Joanna Zajkowska

Department of Infectious Diseases and Neuroinfections, Medical University of Białystok, Białystok, Poland.

Unraveling the link between brain and lymphatic system is result of discovery of lymphatic vessels in the CNS. This is a new insight into communication – the brain and the immune system in cooperation with glimphatic system. System called glymphatic acts largely like the lymphatic system but it is governed by glial cells.

Granting the brain immunological privilege by separating it by the CSF fluid compartment protects our brain from too easy initiation of inflammation in the brain. However, recent discoveries indicate that contact with the immune system exists to a greater extent than the known anatomical structure of the lymphatic system communicating with the brain. The newly discovered lymphatic vessels and the functioning of the glymphatic system indicate ways of eliminating unnecessary substances from the brain. The role of the contact of hidden antigens in the brain with the lymphatic system in the event of their exposure (for example viral infection or teratoma ovarii) is a factor that generates antibodies directed against the structures of the brain and leads to autoimmune encephalitis, an increasingly recognized disease entity. The inflammation directed against NMDR receptors causing anti-NMDR encephalitis is the earliest described. At present, inflammation directed against other surface structures of neurons is diagnosed.

Changes in HSP70 protein expression in MM6 cell line phagocytosing latex beads upon low frequency pulsed electromagnetic field (LF-PEMF; 7 Hz, 30 mT) exposure

Kaszuba-Zwoińska Jolanta[1], Piotr Piszczek[1], Wójcik-Piotrowicz Karolina[2], Guzdek Piotr[3], Gil Krzysztof[1]

1 – Department of Pathophysiology, Jagiellonian University Medical College, Cracow, Poland, Czysta Street 18, 31-121 Cracow, Poland; email: jkaszuba@cm-uj.krakow.pl
2 – Department of Biophysics, Jagiellonian University Medical College, Cracow, Poland, Łazarza Street 16, 31-530 Cracow, Poland
3 – Łukasiewicz Research Network – Institute of Electron Technology, Zabłocie Street 39, 30-701 Cracow, Poland

Introduction: Many studies have demonstrated that electromagnetic fields can exert both stimulatory or inhibitory effect on immune system function, however, precise mechanisms of the interaction are still not elucidated. Long term electromagnetic field exposure can lead to immunosuppression, activation of immune cells and their effector activities or remains without any effect. HSP70 proteins play a key roles in modulation of immune system by ensuring proper folding of proteins and regulation of apoptosis.

Objectives: The aim of the studies was to investigate the effects exerted by low frequency pulsed electromagnetic field (LF-PEMF; 7 Hz, 30 mT) exposure of human monocytic cell line Mono Mac 6, pre-stimulated with inflammatory or proliferatory agents and phagocytosing nanoparticles of latex, on expression of heat shock proteins HSP70.

Materials and Methods: Human monocytic leukemia cell line Mono Mac 6 (MM6) was cultured in RPMI 1640 medium supplemented with fetal calf serum, L-glutamine and gentamicin. MM6 cells were seeded into 96-well plate, stimulated with lipopolysaccharide (LPS), Staphylococcus aureus enterotoxin B (SEB) and phytohemagglutynin (PHA).

After 24 h, latex beads (LB) were added to MM6 cell cultures for phagocytosis and simultaneously LF-PEMF exposure of cells was performed. Following phagocytosis assay, cell cultures were centrifuged and cytosol extracts were prepared for Western blot analysis of HSP70 proteins

Results: The effect triggered by LF-PEMF exposure of MM6 cells pre-stimulated with inflammatory/proliferatory agents like LPS, SEB, PHA and phagocytosing LB, was investigated by Western blot analysis of HSP70 protein expression. Statistical evaluation of the experimental data was performed with U-Mann Whitney and Kruskal-Wallis tests considering $P < 0.05$.

Obtained results are preliminary and show influence of the LF-PEMF exposure exerted on pre-stimulated and phagocytosing latex beads MM6 cells on abundance of HSP70 proteins in cytosolic lysates. LF-PEMF exposure of MM6 cells increased expression of HSP70 proteins in the samples, while the opposite effect was observed in MM6 cell cultures pre-stimulated with LPS or PHA and phagocytosed latex beads. The analogous analysis obtained for SEB agent and LB, has revealed statistically non-significant changes in HSP70 proteins level between samples |exposed/non-exposed simultaneously in LF-PEMF.

Conclusion: LF-PEMF exposure might be a potential modulator of HSP70 protein expression during phagocytosis process, this way influencing innate cellular immune response.

Rhinovirus and coronavirus may infect the lung vascular endothelium – possible implications for immune responses in airway viral infections

Maciej Chałubiński[1], Mateusz Gawrysiak[1], Robert Szewczyk[1], Adrian Gajewski[1], Izabela Gulbas[1], Sylwia Michlewska[2], Aleksandra Likońska[1], Marek L. Kowalski[1]

1 – Department of Immunology and Allergy, Medical University of Łódź, Poland; Pomorska 251 Str., 92-213, Łódź, Poland,
2 – Laboratory of Microscopic Imaging and Specialized Biological Techniques, Faculty of Biology and Environmental Protection, University of Łódź, Poland;Banacha 12/16 Str., 90-237, Łódź, Poland

Vascular endothelium formed by a single layer of squamous endothelial cells of mesenchymal origin regulates the flow of nutrients, cytokines and immune cells between blood and tissues. It may express entry receptors for rhinoviruses (HRV) and coronaviruses (HCoV), and is equipped with pattern recognition receptor (PRR) complex. We postulate that the lung vascular endothelium may be infected by respiratory viruses, sense viral PAMP molecules, including ssRNA and dsRNA, and release biologically active substances. Thus, it may be involved in inflammatory response during airway viral infections. We found that HRV infects human lung microvascular endothelium (HMVEC-L) *via* ICAM-1, activates receptors and pathways of innate immunity (TLR3, TLR7, IRF3, IRF7, RIG-I, MDA5, NOD-2) with subsequent inflammatory and anti-viral cytokine production. It impairs endothelial barrier functions, regenerative capacities, and capability of new vessel formation. Furthermore, HRV enhances a surface expression of HCoV entry receptors: AP-N (229E), DPP4 (MERS), and ACE-2 with TMPSSR2 (SARS-CoV-2), thus increasing a potential susceptibility of lung vascular endothelium to coronaviral infections. Indeed, we observed that HCoV229E may infect HMVEC-L *via* AP-N, induce anti-viral and inflammatory response by activation of TLR- and RLR-dependant pathways, cause strong cytopathic effect and a full damage of endothelial barrier. To conclude, the vascular endothelium of lung vessels has a wide potential to orchestrate inflammation during airway viral infections.

NCN2017/25/B/NZ5/01575

IL-33 as a modulator of the inflammatory and antiviral response of the lung vascular endothelium in rhinoviral infections

Gajewski A. L., Gawrysiak M., Szewczyk R., Kowalski M. L., Chalubinski M.

1 – Department of Immunology and Allergy, Medical University of Lodz, Lodz, Poland

Background: Human rhinovirus (HRV) may cause severe exacerbations of asthma, which is accompanied by increased airway IL-33 concentrations. The answer why rhinovirus is capable of inducing heavy exacerbations in asthmatic patients, whereas in healthy individuals only mild, upper respiratory infections, is still not clear.

Aim: The aim of the study was to analyze the effect of IL-33 on the HRV-induced inflammatory response by the lung vascular endothelium.

Method: Human pulmonary microvascular endothelial cells (HMVEC-L) were exposed to HRV-16 (MOI 3) alone or in the presence of IL-33 (1 and 10ng/mL) for 5, 24 and 72 hours. Number of HRV-16 copies in HMVEC-L, cytokines, chemokines and growth factors mRNA expression were assessed in real-time PCR. Protein concentrations were assessed in BioPlex. The surface expression of ICAM-1 (receptor for HRV) was assessed by flow cytometry.

Results: HRV16 strongly increased the release of plenty of inflammatory cytokines, including IL-1β, IL-6, IL-1Ra, TNF-α, RANTES, IP-10; chemokines: MIP-1α, MIP-1β, IL-8 and eotaxin as well as growth factors: G-CSF, GM-CSF, FGF, VEGF. HRV16 enhanced both mRNA expression and protein release of type I interferons (IFN-β, IFN-λ). It also increased T-type cytokine production: T1: IL-12, IFN-γ; T2: IL-4, IL-5 and IL-13; Th17 – IL-17, but not Treg – IL-10. HRV16 also increased production of IL-2, IL-9, IL-15. IL-33 increased the capture of HRV16 particles associated with the increase of ICAM-1 entry receptor expression. Of inflammatory cytokines, IL-33 increased the HRV16-induced release of IL-1β, IL-6, not RANTES, IP-10, TNF-α. Of chemokines, IL-33 enhanced HRV16-induced production of IL-8, eotaxin, MIP-1α, not MIP-1β, MCP-1. IL-33 enhanced HRV16-induced G-CSF, GM-CSF, FGF and VEGF release. IL-33 up-regulated HRV16-induced T-type cytokines release of T1: IL- 12 (not IFN-γ), T2:IL-4, IL-5 and IL-13, IL-17 – T17, and IL-10 – Treg. Importantly, IL-33 enhanced neither IFN-β, nor IFN-λ release. IL-33 alone increased the release most of the cytokines, e.g: RANTES, IP-10, IL-6, GM-CSF, VEGF, IL-12, IL-4, IL-10, but not IFNs. IL-33 increased the expression of TLR3 and TLR7 as well as IRF3 and IRF7 – intracellular signal transmitters from TLRs, leading to the enhanced production of inflammatory cytokines described above.

Conclusion:IL-33 may augment the effect of rhinovirus HRV16 on inflammatory, but not antiviral, activity of the human lung vascular endothelium, and thus lead to the heavy exacerbations of asthma.

National Science Center 2017/25/B/NZ5/01575.

Acute phase proteins in rabbits in acute viral infection, on the example of GI.1a-RHDVa virus (rabbit haemorrhagic disease virus)

Łukasz Baraniecki[1], Beata Tokarz-Deptuła[1], Andrzej Pępiak[2], Wiesław Deptuła[3]

1 – Institute of Biology, University of Szczecin
2 – Dr Andrzej Pępiak Domestic Animal Clinic, West Pomeranian Veterinary Education Center of Szczecin
3 – Institute of Veterinary Medicine, Faculaty of Biological and Vaterinary Sciences, Nicolaus Copernicus University in Toruń

The immune response in acute viral infections, e.g. infections caused by hemorrhagic fever viruses, is related to the elements that create reactivity of the macroorganism, appearing in the first hours after infection. Among these elements are acute phase proteins (APPs), which so far have been rarely studied in rabbits, while in those animals infected with the highly infectious GI.1a-RHDVa virus causing rapid and acute disease, have not been conducted before. The experiment was carried out on 40 healthy Polish rabbits, which were experimentally infected with GI.1a-RHDVa virus – Dutch strain NL-2 with positive haemagglutination, which caused high mortality and was characterized by an acute course of the disease lasting basically up to 24-72 hours. In the experiment, three groups of 10 infected animals were created and the fourth group of 10 control animals. In infected animals (groups I-III) the concentration of C-reactive protein (CRP) – group I, serum amyloid A (SAA) – group II and haptoglobin (Hp) – group III – was determined. APPs concentration in the blood of groups I-IV was chacked every 12 hours in the first two days (0-48h), and every 4 hours between 48-60 hours, using the USCN Life Science ELISA test. Due to the death of infected rabbits, the CRP protein was determined up to 60 hours (group I), the SAA protein up to 48 hours (group II), and the Hp protein up to 52 hours (group III). It was found that statistically significant differences in CRP protein values were obtained at 36, 48 and 52 hours, SAA proteins at 12, 24 and 36 hours, and Hp proteins at 12, 24, 36 and 48 hours after infection of the animals. The obtained picture of changes in the three examined APPs shows that they are important indicators in rabbits in this infection, and the picture of these changes proves that not only the CRP protein plays an important role in these animals, as it has been assumed so far, but also proteins SAA and Hp play key role.

15. Immunology of Reproduction

Molecular basis of male infertility; systemic biology approach

Maciej Kurpisz[1], Agnieszka Malcher[1], Piotr Jędrzejczak[2]

1 – Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland
2 – Infertility and Reproductive Endocrinology Clinic of Gynaecological and Obstetric Hospital, University of Medical Sciences, Poznan, Poland

Background: Genes of immunological system seem to play important functions within a semi-privileged male gonad. They may also potentially act as predictive factor for molecular diagnosis, treatment and monitoring of azoospermia syndrome.

Methods: We have analyzed 20 testicular biopsy samples with Affymetrix Human Gene 1.0 ST microarrays – sixteen were obtained from patients with various types of non obstructive azoospermia (NOA) syndrome and four were with normal spermatogenesis. Six of the patients with NOA syndrome were subjected to gonadotropin therapy (hCG+rFSH) and one positive responder was re-analyzed in microarrays before and after successful treatment. Genes analyzed by microarrays were validated, alleles determined and sequenced. All patients subjected to experimental therapy undergone histocompatibility genes expression evaluation.

Results: Conclusions originating from transcriptomic studies are the following. Among the 5,000 significantly different gene expression in infertile vs healthy individuals (gonad), 14 were delineated with the highest range of expression from the background. There were 7 genes which were most significantly downregulated (*AKAP-4, UBQLN3, CAPN11, GGN, SPACA4, SPATA3, FAM71F1*) and 7 significantly upregulated (*WBSCR28, ADCY10, TMEM225, SPOATS1, FSCN3, GTSF1L, GSG1*). According to positive versus negative responders of NOA patients to gonadotropin therapy, 5 transcripts were found to be significantly different. Among them, belonging to Class II HLA DQB1 acquired statistical power to differentiate between successful and negative therapy. Additionally, in collaboration with University of Pittsburgh we have identified novel *TEX11* gene of which mutations occurred in 15% of males with meiotic type of azoospermia.

Conclusions: The microarray analysis performed demonstrated high utility prognostic value concerning negative correlation between Class II HLADQB1 expression and successful gonadotropin therapy. Increase of Class II expression in male gonad may evidence presence of cells related to bone marrow origin. Possible impact of this phenomenon on male infertility diagnosis, prognosis and therapy will be discussed.

This study was funded by the Polish National Science Centre (no 2020/37/B/NZ5/00549)

The tolerogenic function of regulatory lymphocytes in pregnancy – receptors and ligands

Anna Chełmońska-Soyta[1,2], Anna Kędzierska[1], Daria Lorek[1], Anna Sławek[1], Paulina Sypek[1]

1 – Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland
2 – Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland

The presence of an antigenically foreign foetus induces a state of immune tolerance in the mother organism that is crucial to embryo implantation and foetus development. Imbalances in immune tolerance may cause a variety of reproductive failures such as preeclampsia or spontaneous and recurrent miscarriage. It is estimated that the problem of miscarriage affects one in four recognized pregnancies, with 85% of them being lost in the first trimester

The exact mechanisms associated with natural tolerance during pregnancy is still unknown. Nevertheless, it is showed that among cells that contribute to the development of tolerance to foetal antigens are regulatory T lymphocytes (Tregs) and regulatory B lymphocytes (Bregs). In our studies we showed that classical tregitopes – epitopes derived from immunoglobulins for TCR receptors of Treg cells are able to counteract foetal resorption and restore not only the concentration of Treg lymphocytes but also B cells producing IL-10 at periphery and in uteral lymph nodes. Similarly new, designed by us peptides – epitopes derived from other mammalian proteins and also of bacterial origin are able to increase the pole of Treg and Breg cells and restrict foetal resorption in abortion prone model in mice. Tregitopes changed DC and B cell costimulatory phenotype towards tolerogenic one.

More evidences indicate also that Breg lymphocyte may be a crucial cell in the development of pregnancy tolerance. Another our studies showed that early pregnancy recognition and tolerance development may be regulated by TLRs present on B cells and IL-35 produced by Bregs which promotes Tregs expansion. All above presented results indicate that at least in mice pregnancy success is dependent on mutual interaction between T and B regulatory lymphocytes. Putative therapeutic intervention focused on modulation the number and effector function of these cells may be one of the possible ways of treatment of pregnancy related reproductive failure.

Role of peritoneal fluid in the immunopathogenesis of endometriosis

Jacek Malejczyk

Chair and Department of Histology and Embryology, Medical University of Warsaw, Warsaw, Poland. jacek.malejczyk@wum.edu.pl

Endometriosis is a common gynecological disorder related to the presence of endometrial-like tissue outside uterine cavity. It manifests in peritoneal inflammation, pelvic pains and subfertility. Endometriosis is also associated with local and systemic abrogation of the immune system displaying features of an autoimmune disorder. These include increased prevalence of various autoantibodies, disturbance of Treg cells, disturbance of Th1/Th2 cell balance, and abrogated NK cell activity. The mechanisms underlying these deviations still remain poorly understood. We and others found that peritoneal fluid of women with endometriosis contains increased amounts of various immunoregulatory cytokines and chemokines. Of particular interest may be IL-10, TGF-β, and CCL20 which were found to be associated with increased frequency of CD4+ CD25+ FOXP3+ Treg cells. The latter one appears to be responsible for attraction and migration of Treg cells from the periphery into the peritoneal cavity. Furthermore, we found that the peritoneal fluid from patients with endometriosis downregulated production of IL-2, IFN-γ, IL-17A, and TNF while increasing IL-4 and IL-10 expression by cultured CD4+ T cells thus suggesting their shift toward the Th2 phenotype. It also stimulated CCL-2 (MCP-1) and inhibited CCL5 (RANTES) and CXCL9 (MIG) as well as stimulated generation of Treg cells and inhibited cytotoxic activity of NK cells thus further confirming its multidirectional inhibitory activity toward cell-mediated type 1 immune responses. In conclusion, the peritoneal milieu in patients with endometriosis exerts immunosuppressive activity on cell-mediated immunity and stimulates Th2 response. This may facilitate implantation and survival of endometriotic tissue as well as may accounts for other endometriosis-associated pathogenic phenomena such as local fibrosis and adhesion formation.

Our studies were supported by the Polish National Science Centre grant no. 2014/13/B/NZ6/00806

The role of ERAP, KIR and HLA-C profile in patients undergoing *in vitro* fertilization embryo transfer

Karolina Piekarska[1], Paweł Radwan[2,3], Agnieszka Tarnowska[1], Rafał Krasiński[2], Michał Radwan[2,4], Andrzej Malinowski[5], Jacek R. Wilczyński[6], Andrzej Wiśniewski[1], Izabela Nowak[1]

1 – Laboratory of Immunogenetics and Tissue Immunology, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Department of Clinical Immunology, Wrocław, Poland
2 – Gameta Hospital, Department of Reproductive Medicine, Rzgów, Poland
3 – Biogeno – Regional Science-Technology Centre, Podzamcze, Poland
4 – The State University of Applied Sciences in Płock, Faculty of Health Sciences, Płock, Poland
5 – Polish Mothers' Memorial Hospital–Research Institute, Department of Surgical, Endoscopic and Oncologic Gynecology, Łódź, Poland
6 – Medical University of Łódź, Department of Surgical and Oncological Gynecology, Łódź, Poland

Background: Endoplasmic reticulum aminopeptidases ERAP1 and ERAP2 trim peptides to the optimal length for binding to HLA class I molecules. HLA-C molecules and its C1 or C2 epitopes are expressed by invading trophoblast and may interact with killer immunoglobulin-like receptors (KIR) on the decidual NK cell surface, therefore ERAP-KIR-HLA-C interactions can impact on implantation failure. We studied the role of genes polymorphism of ERAPs, KIR and HLA-C in 496 women, who undergone *in vitro* fertilization embryo transfers (IVF-ETs) and 385 fertile women with healthy born children after natural conception. We also measured the concentration of ERAP1 and ERAP2 in patients plasma before and after IVF-ETs, and in fertile women.

Methods: Among patients, 283 women were RIF (recurrent implantation failure; mean 4 ETs; range 3-15), 161 women were with clinical pregnancy (mean 1 or 2 ETs; range 1-3). We used the TaqMan SNP Genotyping Assays for *ERAP1* (rs30187, rs27044, rs26653, rs2287987, rs26618, rs6861666) and *ERAP2* (rs2248374) typing. *KIRs* and *HLA-C* were genotyped by PCR-SSP methods. The concentration of ERAPs was tested with a Sandwich enzyme-linked immunosorbent assay kit. Statistical analyses were performed using two-tailed Fisher exact test and Mann-Whitney test (GraphPad Prism 5 software).

Results: Combination of TelAA KIR genotype and HLA-C2C2 was associated with RIF ($p = 0.002$, $OR = 2.39$). This effect was stronger in women with CenAB/TelAA/C2C2 genotype ($p = 0.006$, $OR = 3.43$). We found also the association of rs26653CG genotype in patients positive for HLA-C2C2 group ($p = 0.006$, $OR = 2.73$) in general with infertility. This effect deepened in the RIF group ($p = 0.002$, $OR = 3.66$). Patients positive for ERAP1 rs2287987CT and KIR Tel BB were in higher frequency in fertile women than in IVF group ($p = 0.0008$, $OR = 0.11$). We observed also other weaker associations of ERAPs with HLA-C and KIR polymorphisms.

Moreover, we observed secreted ERAP1 in plasma of fertile women, but we didn't detect ERAP1 in IVF patients plasma. IVF patients secreted higher concentration of ERAP2, than fertile women who have healthy born children ($p = 0.02$). Even stronger significance ($p = 0.0035$) was obtained when embryo transfer resulted in miscarriage.

Conclusion: Fertile women differ in *ERAP-KIR-HLA-C* genetic profile and ERAP1/ERAP2 secretion from women participating in IVF.

This study was funded by the Polish National Science Centre (no. 2014/13/B/NZ5/00273 and 2018/29/N/NZ5/00940).

16. Immunomodulation and Immunotoxicology

Immunomodulatory effect of xenobiotics

Wioletta Ratajczak-Wrona

Department of Immunology Medical University of Bialystok, Poland

The progressive development of civilization, in addition to the improvement of the standard of living, has led to the contamination of the environment with synthetic substances that may adversely affect the health of humans. Of particular importance among the xenobiotics are xenoestrogens, which are endocrine active compounds (EDCs) capable of altering the functions of the endocrine system. This group includes bisphenols, parabens, organochlorine compounds, synthetic estrogens, and alkylphenols. Due to their widespread use in industries, as well as their presence in many products used on a daily basis, almost the entire population is at the risk of exposure to EDCs, regardless of the type of occupation or the place of residence. EDCs are present in food, water, or cosmetics, from which they are easily absorbed into the human body. They also have the ability to modulate the immune system by influencing the nonspecific and specific response mechanisms, and can disrupt maturation, cellular and humoral activity, and cell survival. As EDCs show a nonmonotonic dose–effect relationship, depending on the concentration, as well as sex, exposure time, and route of administration, they may exert an immunosuppressive effect on the immune system. Additionally, these compounds can possibly accumulate in the body from various products. Daily exposure to EDCs, even at low concentrations, may cause systematic weakening of the immune system, which in the long run, in the face of a real threat in the form of contact with various pathogens, may result in impaired defense mechanisms.

Role of monocytes in response to air pollution

Jarosław Baran

Department of Clinical Immunology, Jagiellonian University Medical College, Cracow, Poland

In recent years, the air pollution has significantly contributed, among others, to the increase in incidence of allergic and autoimmune diseases. Many studies suggest that transition metal containing particulate matter (TMCPM) may play a role in initiation and development of these disorders. In this context, we asked whether TMCPM, a component of air pollution, has any effect on antigen presenting capacity and pro-inflammatory response of peripheral blood monocytes.

Monocytes were obtained from peripheral blood mononuclear cells of healthy blood donors by countercurrent centrifugal elutriation and cultured *in vitro* with or without TMCPM. Two different preparations of TMCPM were used in the study: NIST (SRM 1648a- standard urban particulate matter obtained from US National Institute for Standards and Technology) and LAP- SRM 1648a particulate matter treated within 120 min with cold oxygen plasma for the removal of organic compounds from the reference material. Antigen presenting capacity of monocytes, alterations in their morphology and viability, as well as the production of pro-inflammatory cytokines (IL-1β, IL-6, IL-8, TNF) and reactive oxygen species (ROS), mitochondrial membrane potential, Caspase-9, Caspase-3, and Caspase-1 activation were examined after the exposure of monocytes to TMCPM.

We have detected that TMCPM treatment affects antigen-presenting capacity of monocytes to autologous T cells. Investigating mechanism of this phenomenon, we showed alterations in monocyte morphology and their viability. This was associated with an increased production of ROS, leading to the disruption of mitochondrial membrane potential, activation of Caspases-9 and Caspase-3, and monocyte cell death. Moreover, the increased production of pro-inflammatory cytokines by monocytes and Caspase-1 activation rapidly after their exposure to TMCPM**,** followed by IL-1β production was detected, suggesting the involvement of inflammasome activation in this process.

In conclusion, TMCPM has effect on biological functions of monocytes and induces their strong inflammatory response, suggesting their role in mechanism of air pollution-induced diseases.

This study was supported by grant from the National Science Centre (NCN) in Poland (grant APARIC no. 2015/16/W/ST5/00005).

The influence of selected bioelements on immune system function

Sylwia Terpiłowska[1], Andrzej K. Siwicki [2]

1 – Institute of Health Sciences, Faculty of Natural Sciences and Health, The John Paul II Catholic University of Lublin, Racławickie 14 Av., 20-950 Lublin, Poland
2 – Department Microbiology and Clinical Immunology, Faculty of Veterinary Medicine, University Warmia and Mazury in Olsztyn, Oczapowskiego 13 Str., 10-957 Olsztyn, Poland

Bioelements are divided on macroelements and microelements. Elements that human or animals need in large amounts to live are known as macroelements. Mineral elements that are needed by human or animals in only trace amounts are known as microelements. Micro- and macroelements get into the bodies of animals and people through the respiratory tract, through skin and to the greatest extent through the digestive tract. They get together with the diet, but also in the form of vitamin and mineral preparations, dietary supplements or energy drinks, supply of which has recently increased significantly. In recent years, more and more elements have been introduced into human organisms along with large-scale biomaterials. The released metal ions form biometerials may cause type IV inflammatory and hypersensitivity reactions, and alternations in bone modeling that lead to aseptic loosening and implant failure. The ions of metals released from the surface of the implant are absorbed by macrophages which are involved in many of the processes associated with phagocytose orthopaedic biomaterials particles and release the pro-inflammatory mediators. These cytokines such as IL-1 α and β stimulate resorption of bone and then they act synergistically to the TNF-α. Moreover, macrophages release matrix metalloproteinases and chemokines Another investigation has shown that Cr and Co ions inhibitis osteoblasts, osteoclasts and T and B cell proliferation.

A number of elements have the ability to modulate immune response through the production of antibodies or cytokines (eg Zn, Se, Cr). Moreover the elements are required for immune cells proliferation or activation (eg Iron). What is more, immune cells and their mechanisms of phagocytic activities are affected by microelements deficiencies. It has been proved that selenium supplementation improves neutrophil's phagocytic capacity. However, low copper status reduces neutrophil phagocytic capacity. Moreover, it has been shown that selenium deficiency affects blood levels of IgG, IgM and IgA as well as T cell function.

The elements are also required for functioning antioxidant system (eg Se) of the immune cells. On the one hand, they can be expected to increase the production of reactive oxygen species. On the other hand, trace elements are involved in the antioxidant system and the deficiency of any of them may depress immunity. Uncontrolled oxidation reactions may impair the animal's immune status.

Parabens – endocrine disrupting chemicals (EDCs) action on immune cells

Karolina Nowak[1], Ewa Jabłońska[1], Marzena Garley[1], Piotr Radziwon[2], Wioletta Ratajczak-Wrona[1]

1 – Department of Immunology, Medical University of Bialystok, Poland
2 – Regional Centre for Transfusion Medicine, Bialystok, Poland

Background: One of the most commonly used preservatives of food, cosmetics and pharmaceutics are parabens e.g., methyl- (MeP), ethyl- (EtP), propyl- (PrP) and butylparaben (BuP). It is highly concerning that parabens were regarded as endocrine disrupting chemicals (EDCs). Scientists underlined its ability to mimicking of 17β-estradiol (E2) via modulation of estrogen receptor (ER)α/β signaling. ERs are present in immune cells including neutrophils, which play a crucial role in inflammation and fighting against bacteria, fungi and cancer cell invasion. According to our previous research, neutrophil functioning (chemotaxis, phagocytosis and oxygen-dependent mechanism of killing) was disrupted in MeP-exposed cells. So far, parabens' impact on the oxygen-independent mechanism of killing including production of serine proteases (neutrophils elastase and proteinase 3) was not investigated.

Aim: The study aimed to investigate impact of parabens (MeP, EtP, PrP and BuP) and E2 on serine proteases (neutrophils elastase and proteinase 3) expression, as well as the ER-dependent mechanism of parabens action in female and male neutrophils.

Material and methods: Material to study were blood donated by healthy women (n=4) and men (n=4). Isolated neutrophils were cultured for 2 hours without or with MeP, EtP, PrP, BuP or E2. The expression of neutrophils elastase, proteinase 3, ERα, ERβ and β-actin were investigated by western blot method. Results were analyzed in Statsoft Statistica.

Results: Exposure to E2 increases expression of proteinase 3, ERα and ERβ in female cells, as well as expression of ERβ in neutrophils from men in comparison to non-exposed cells. Expression of neutrophil elastase increased in female neutrophils exposed to PrP and BuP in comparison with non-exposed cells, while in male neutrophils its expression increased after incubation with all tested parabens. Expression of proteinase 3 increased in female neutrophils exposed to EtP, PrP and BuP, whereas, in male cells expression of protein increased only after incubation with PrP and BuP. In female neutrophils, which was not exposed or incubated with EtP and E2, the expression of proteinase 3 was higher than in male neutrophils. Expression of both ERs in neutrophils incubated with parabens was not changed in comparison with cells incubated without preservatives.

Conclusions: Based on our results we suggest that parabens activate serine proteases in human neutrophils; its modulation is dependent on length of parabens substituent and sex. Parabens' way of action is different than E2 and is independent of ERs.

This study was funded by the National Science Centre, Poland (grant no. 2018/31/N/NZ7/01074).

Particulate matter of air pollution promotes proinflammatory activity of Th lymphocyte subsets

Adrianna Gałuszka[1], Małgorzata Stec[1], Kazimierz Węglarczyk, Anna Kluczewska[1], Maciej Siedlar[1], Jarosław Baran

Department of Clinical Immunology, Institute of Pediatrics, Jagiellonian University Medical College, Wielicka Street 265, 30-663 Cracow, Poland

In recent years, an increase in air pollution including transition metal containing particulate matter (TMCPM) seriously impacts human health. However, the knowledge on the effects of TMCPM on the cells of immune system is still scarce. Here we asked whether TMCPM has effect on the activity of human CD4+ T cell subsets (Th1, Th2, Th17, and Treg).

Peripheral blood mononuclear cells (PBMC) of healthy donors were isolated and cultured with or without TMCPM: NIST (SRM 1648a- standard urban particulate matter obtained from US National Institute for Standards and Technology) and LAP-SRM 1648a particulate matter treated within 120 min with cold oxygen plasma for the removal of organic compounds. As a positive control, PBMC were stimulated with PMA (Phorbol Myristate Acetate) and ionomycin. In some experiments, T cells (lymphocyte fractions from counter-current centrifugal elutriation) or T cells added to monocytes, exposed to TMCPM pre-treated or not with Polymyxin B at the concentration of 100 µg/ml were used. After 5 hours of culture, the expression of intracellular proteins (IFN-γ, IL-4, IL-17 and Foxp3) was analyzed by flow cytometry.

The results showed that treatment of PBMC with TMCPM increased expression of IFN-γ and IL-17A, specific for Th1 and Th17 cells, respectively. Moreover, a decrease in the expression of Foxp3 (Treg) was noticed, while the frequency of cells positive for IL-4 (Th2) was negligible. The observed effect was dose dependent, being most pronounced at the highest concentration of NIST (100µg/ml), containing more organic components e.g. LPS (lipopolysaccharide) and required the presence of monocytes. In addition, inactivation of endotoxin (LPS) by treatment of TMCPM with polymyxin B did not change the activity of Th subsets in the presence of monocytes, suggesting also a role of their inorganic components.

In conclusion, results indicated that in vitro treatment of human PBMC with TMCPM skews the balance of Th1/Th2 and Treg/Th17 cells, promoting pro-inflammatory activity of the Th1 and Th17 subsets by monocyte activation in organic and inorganic compounds dependent manner. This observation may confirmed the hypothesis that TMCPM play role in the development and exacerbation of allergies, and inflammatory and autoimmune disorders.

This study was supported by grant from the National Science Centre (NCN) in Poland (grant APARIC no. 2015/16/W/ST5/00005) and by H2020-MSCA-RISE-2016 project "CHARMED" no. 734684.

Coanalgesics modulate tramadol-induced immune effects in mice

Iwona Filipczak-Bryniarska[1], Katarzyna Nazimek[2], Michael Kozlowski[1], Krzysztof Bryniarski[2]

1 – Department of Pain Treatment and Palliative Care, Jagiellonian University Medical College, Krakow, Poland
2 – Department of Immunology, Jagiellonian University Medical College, Krakow, Poland

Background: Macrophages (Mf), involved in the pathogenesis of pain, express a variety of receptors enabling responsiveness to certain medications, including opioids and coanalgesics (CAs). Analgesic effects of the latter are likely associated with immunomodulatory activity that remain not fully understood. Thus, current research aimed at examining the impact of CAs (acetaminophen and dexketoprofen) on tramadol-induced effects exerted on mouse immunity.

Methods: Macrophages from mice treated with tramadol with or without CAs, were cultured to evaluate generation of nitric oxide or reactive oxygen intermediates (ROIs), pulsed with either corpuscular antigen (sheep erythrocytes, SRBC) or hapten (trinitrophenyl, TNP) and transferred to naive recipients to induce humoral or contact hypersensitivity (CS) responses, respectively. Active contact hypersensitivity was also elicited in drug-treated mice.

Results: We observed that repeatedly administered tramadol and CAs in all combinations increased the production of ROIs and NO in the presence of zymosan or LPS, respectively. Further, acetaminophen in the presence of tramadol enhanced SRBC-Mf-activated humoral response measured in plaque forming assay (PFA), in contrast to dexketoprofen, which seemed to accelerate the immunoglobulin class-switching process. Finally, tramadol in the presence of acetaminophen or dexketoprofen inhibited active CS reaction in mice as well as CS mediated by Mf as APC.

Conclusions: Our study demonstrated modulatory activity of CAs on humoral immune response and CS reaction across the broad spectrum of macrophage immune functions, which is likely critical to their activity supporting the beneficial effect of tramadol in the pain treatment.

Acknowledgments: This study was supported by Polish Ministry of Science and High Education grant No N41/DBS/000073.

Phagocytic activity of the monocyte-macrophage cell line (MM6) stimulated with infection agent (LPS or SEB) and exposed to low-frequency pulsed electromagnetic field (LF-PEMF; 7 Hz, 30 mT) analyzed by flow cytometry method

Piotr Piszczek[1], Bernadeta Nowak[2], Karolina Wójcik-Piotrowicz[3], Piotr Guzdek[4], Krzysztof Gil[1], Jolanta Kaszuba-Zwoińska[1]

1 – Department of Pathophysiology, Jagiellonian University Medical College, Czysta street 18, Krakow, 31-121, Poland;
2 – Department of Immunology, Jagiellonian University Medical College, Czysta street 18, Krakow, 31-121, Poland;
3 – Department of Biophysics, Jagiellonian University Medical College, Łazarza street 16, Krakow, 31-530, Poland;
4 – Łukasiewicz Research Network - Institute of Electron Technology, Zabłocie street 39, 30-701, Krakow, Poland.

One of the crucial effector mechanisms in innate immunity responsible for destruction of evading pathogens is phagocytosis. There are a lot of experimental studies showing that electromagnetic fields exposures influence effector activity of immunocompetent cells.

Aim of the study: The aim of the study was to research the effect exerted by extremally low frequency electromagnetic fields (LF-PEMF) exposure on the phagocytic activity in cells of the human monocyte-macrophage cell line – MM6 – stimulated with lipopolysaccharide (LPS), Staphylococcal enterotoxin (SEB) and phytohemagglutinin (PHA).

Methods: Mono Mac 6 (MM6) human cell line was cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and gentamicin-glutamine solution, at the temperature of 37°C, 95% air humidity atmosphere and of 5% CO2 content, in 96-well plates. The experiments were performed in the phase of cells logarithmic growth. MM6 cell cultures have been seeded at the density 1×106 cells per milliliter for experiments. After 24 hours since the experiment has been set up, the cells were stimulated by LPS, SEB or PHA at final concentration 1 µg/ml. Next day, phagocytosis assay was carried out by adding fluorescent orange B stained latex beads to cell cultures treated with LPS, SEB or PHA. During phagocytosis assay cell cultures were exposed to LF-PEMF (7 Hz, 30 mT) by 3 hours. Phagocytosis was stopped by putting cell culture plates on ice. Cells were harvested, centrifuged, washed twice with cold PBS and resuspended in PBS for flow cytometry (FACS Calibur) analysis. FL-1 fluorescence resulting from phagocytic activity of cells was measured and expressed as percentage of positively stained cells. Obtained results were statistically analyzed by the Student's t-test ($P < 0.05$) and expressed as mean (+) standard deviation.

Results: There were no statistically significant differences in phagocytosis activity in cells stimulated with LPS, SEB or PHA in the samples exposed to LF-PEMF comparing to the samples stimulated with LPS, SEB, PHA and non-exposed to LF-PEMF. There were also no differences between the series non-stimulated with the infectious agent or PHA and exposed to LF-PEMF and the samples without LPS, SEB or PHA and without LF-PEMF exposure.

Conclusions: LF-PEMF exposure does not enhance in vitro phagocytosis activity of the MM6 cells stimulated by infectious or proliferatory agent like LPS, SEB or PHA. What more LF-PEMF doesn't increase phagocytosis of latex nanoparticles in MM6 cells non-stimulated by any inflammatory or proliferatory agent modulating the phagocytic activity of MM6 cells.

Regulation of NLRP3 inflammasome expression by diosgenin in human neutrophils

Iwaniuk Agnieszka[1], Ratajczak-Wrona Wioletta[1], Garley Marzena[1], Jabłońska Ewa[1]

1 – Department of Immunology, Medical University of Bialystok, J.Waszyngton 15A, 15-269 Bialystok, Poland.

Background: Neutrophils, PMNs, are the first line of defense against pathogenic microorganisms. They are also the main source of pro-inflammatory cytokines such as IL-1β. The mechanism of IL-1β secretion is mediated by inflammasomes – multi-protein complexes formed with the participation of recognize molecular patterns receptors. The best known and described inflammasome is the complex associated with the NLRP3 receptor. Its formation occurs by attaching the ASC adapter protein and caspase-1 (p-20). This results in the activation of pro-IL-1β – pro-inflammatory cytokine into their active form. Substances with immunomodulatory properties that would regulate the inflammatory process, and at the same time be a natural, easily available dietary supplement are in demand. An example of such a substance is diosgenin. It is a representative of steroidal saponins belonging to triterpenes, found in plants of the Dioscoreaceae family.

Aim of the study: The aim of the study was to assess the expression of proteins involved in the formation of the NLRP3 inflammasome, as well as to study its activity by assessing pro-inflammatory IL-1β expression after exposure to diosgenin.

Materials and methods: The studies were carried out on neutrophils isolated from the blood of 10 healthy, voluntary donors. PMNs were incubated for 1 hour with diosgenin (10 μM), then cells were activated with LPS and ATP. The effect of diosgenin on the expression of NLRP3, ASC, caspase-1 and IL-1β proteins was assessed using the Western Blot method. IL-1β secretion in PMNs supernatants was assessed by ELISA

Results: Studies have shown a decrease in the expression of NLRP3 inflammasome proteins such as NLRP3, ASC and caspase-1 after exposure of PMNs to diosgenin, compared to unstimulated PMNs. A similar direction of changes was observed in PMNs after incubation with diosgenin and activated with LPS + ATP compared to activated PMNs only. A lower expression of IL-1β was also demonstrated in diosgenin treated PMNs as well as diosgenin incubated and activated PMNs, compared to unstimulated and LPS+ATP activated PMNs, respectively.

Conclusion: The above studies indicate the inhibitory effect of diosgenin on the forming the inflammasome NLRP3 proteins and the secretion of IL-1β in human neutrophils, indicating to a new aspect of anti-inflammatory activity of diosgenin.

The impact of endurance effort on T helper cell subsets distribution in peripheral blood of young men

Dorota Kostrzewa-Nowak, Robert Nowak

Centre for Human Structural and Functional Research, Institute of Physical Culture Sciences, University of Szczecin, 17C Narutowicza St., 70-240 Szczecin, Poland

Changes in distribution of Th1 and Th2 cell subsets as a consequence of the post-exercise cytokine secretion of participants and professional athletes in different age groups favors the emergence of type 2 cell subsets. The participation of these cells leads not only to local immune changes, but may also underlie the post-effort modulation of the immune response at the systemic level. The aim of this study was to assess the impact of endurance effort on Th cell subset distribution and the post-effort changes in cytokine levels related to Th cells.

Sixty-two males, median aged 17 years old (range 16–29 years) were divided into groups performing the endurance effort, according to the YO-YO intermittent recovery test level 1 (YYRL1) and the maximal multistage 20 m shuttle run (Beep). Blood samples were taken three times: at baseline, post-effort, and in recovery. To determine percentages of studied Th cell subsets the Human Th1/Th2/Th17 and Th17/Treg Phenotyping Kits were used. Also selected cytokines concentration, namely IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-17A, TNF-α and IFN-γ were performed using BD Cytometric Bead Array.

The percentage of Th1 cells increased in post-effort and recovery time point. The post-effort percentage of Th1 cells was higher in the Beep group compared to the YYRL1 group. Significant post-effort increase in Th17 cells was observed in both groups. The post-effort percentage of Treg cells increased in the Beep group. An increased post-effort concentration of IL-2, IL-6, IL-8 and IFN-γ in both groups was observed. Post-effort TNF-α and IL-10 levels were higher than baseline in the YYRL1 group, while the post-effort IL-17A concentration was lower than baseline only in the Beep group. The recovery IL-2, IL-4, TNF-α and IFN-γ levels were higher than baseline in the YYRL1 group. The recovery IL-4, IL-6, IL-8, TNF-α and IFN-γ values were higher than baseline in the Beep group.

The molecular patterns related to cytokine secretion are not the same for different protocols of progressive effort. The immune response induced by endurance effort causes an increase in Th1 cells, while only the effort on athletic tracks induces an increase in Th17 cells. The progressive effort induces both Th1- (IL-2, TNF-α, IFN-γ) and Th2-related (IL-4, IL-6) cytokine release. It seems that Treg cells are probably the key cells responsible for the silencing the inflammation and enhancing the anti-inflammatory pathways.

Analgesic adjuvants facilitate morphine anti-inflammatory activity in mice

Michael Kozlowski[1,2], Katarzyna Nazimek[2], Iwona Filipczak-Bryniarska[1], Krzysztof Bryniarski[2]

1 – Department of Pain Treatment and Palliative Care, Jagiellonian University Medical College
2 – Department of Immunology, Jagiellonian University Medical College

Background: Macrophages are a versatile group of cells fulfilling crucial roles in both the innate and adaptive immune responses. Macrophages also express a variety of surface receptors enabling them to respond to certain medicaments, including those used in pain treatment. Adjuvant analgesics (AAs) are effective in specific clinical situations, including neuropathic pain. Their analgesic effects are likely associated with immunomodulatory activities, that are not well explored. Thus, current research aimed at examining the impact of AAs on morphine effects on macrophages during innate and adaptive responses in mice.

Methods: Macrophages from mice treated with morphine with or without an analgesic adjuvant (gabapentin, amitriptyline, or venlafaxine) were either subjected to reactive oxygen intermediates chemiluminescence assay, cultured to evaluate the generation of cytokines, or were pulsed with either corpuscular antigen or hapten and transferred to naive recipients to induce humoral or cellular response, respectively. Active contact hypersensitivity was also elicited in drug-treated mice. Phagocytosis assay was performed in the case of mice treated with morphine with or without gabapentin, amitriptyline or venlafaxine.

Results: We observed that repeatedly administered morphine and analgesic adjuvants reduced antigen phagocytosis by macrophages. Analgesic adjuvants tended to decrease the production of reactive oxygen intermediates and nitric oxide, with amitriptyline having the greatest effect. Further, amitriptyline with morphine enhanced basal secretion of cytokines by macrophages, and all analgesic adjuvants tended to decrease LPS-stimulated release of pro-inflammatory cytokines. Morphine and analgesic adjuvants impacted the expression of phagocytosis and antigen-presentation markers on macrophages, which led to the reduced ability of morphine-affected macrophages to induce B-cell secretion of specific antibodies, and the addition of AAs strengthened this effect. Finally, gabapentin and venlafaxine suppressed the contact hypersensitivity reaction, while amitriptyline seemed to have the opposite effect.

Is fever important for ME-induced immune response? *In vitro* studies

Kozłowski Henryk[1], Wrotek Sylwia[1]

1 – Department of Immunology, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Toruń

Mistletoe Extract (ME) has a long history of about 100 years as an add-on therapy for cancer treatment in German-speaking countries. Usually it is applied for managing side effects during anticancer therapy but research revealed that ME possesses also anticancer properties and exerts immunomodulatory effects. Interestingly in an observational study on a large group of ME-treated patients, fever was a common reaction. This observation is often ignored in *in vitro* studies on ME and heat is usually not taken into account. Therefore the aim of this study is to investigate ME-triggered effects on macrophages that are cultured in fever-ranged temperatures.

The RAW264.7 cells were simultaneously stimulated with ME and subjected to fever-range hyperthermia (FRH; 39°C or 41°C). After co-treatment, cell viability was measured by MTT assay. Additionally, the generation of reactive oxygen species (ROS) was evaluated using carboxy-2′-7′-dichlorodihydrofluorescein diacetate (carboxy-DCF_DA) followed by flow cytometry. The cell cycle distribution was analysed by propidium iodide staining and flow cytometry. Finally, the production of pro-inflammatory factors (interleukin (IL)-1β, IL-6, and cyclooxygenase (COX)-2) was measured using two-step RT-qPCR.

The results showed significantly inhibited viability in ME-treated RAW264.7 cells cultured at 37°C. This effect can be abolished by elevation of the temperature to 39°C or 41°C. ME-treated cells cultured at 37°C did not reveal any significant change in ROS level. Interestingly, ME-treated cells exposed to 41°C display two times higher ROS generation than control cells. ME treatment did not show any changes in cell cycle distribution, nevertheless elevation of ambient temperature to 39°C induced in ME-treated cells a cell cycle arrest in the G1 phase. Furthermore, heat treatment demonstrated an increase in the number of G2/M cells cultured at 41°C. Exposure of RAW264.7 cells to ME did not change mRNA expression of IL-1β, IL-6, and COX-2 in cells cultured at 37°C. However, in cells cultured at 41°C, we observed a significant increase in all pro-inflammatory factors mRNA expression.

Our research suggest that fever is an important component of ME-induced immune response and should be taken into account during *in vitro* research. Soon, we are going to confirm these interactions on additional monocyte cell line J774.

The usefulness of interleukin 6 in assessing the biocompatibility of polymer composites for dental prosthetics

Agnieszka Żydek, Anna Maliszewska, Anna Mertas

Department of Microbiology and Immunology, Faculty of the Medical Sciences in Zabrze, Medical University of Silesia in Katowice

Backgroud: Interleukin 6 (IL-6) is known as pro-inflammatory cytokine, produced in response to a damaging factor. The aim of the study was to evaluate the release of IL-6 by human gingival fibroblasts *in vitro* contacted with extracts of a new polymer composite (polydimethylsiloxane-based material with silver sodium hydrogen zirconium phosphate at concentrations of 2, 4, 6, 8, 10, 12, and 14% w/w as an antimicrobial filler) to be used as a soft denture lining material.

Methods: Human gingival fibroblasts cel line HGF-1 (ATCC, Manassas, VA, USA) was used in our *in vitro* studies. In the first stage of the study, the MTT test was performed. This test was provided to determine the viability of HGF-1 cells contacted *in vitro* for 24 and 48 hours with extracts of the tested polymer composites in culture conditions. An ELISA method (Human IL-6 ELISA Kit, Diaclone, France) was used to determine in culture supernatants the concentration of IL-6 released by HGF-1 cells contacted *in vitro* with the tested extracts. The obtained results were subjected to statistical analysis using the Statistica 13 program (StatSoft, Poland).

Results: The results of the MTT test, interpreted in accordance with the requirements of PN-EN ISO 10993: 2009, confirmed the lack of cytotoxic activity of the tested polymer composite with different content of nanofiller with antimicrobial properties. In all tested cell culture supernatants, no statistically significant increased release of IL-6 by cells contacted with the tested extracts was found, compared to the control cell culture.

Conclusion: The obtained results of performerd *in vitro* tests confirm the lack of cytotoxicity of the tested polymer composites with antimicrobial filler and prove that studied material does not induce an inflammatory reaction**.**

Does the maximal progressive effort carried out according to various protocols trigger immunomodulatory processes according to the same molecular pattern?

Robert Nowak, Dorota Kostrzewa-Nowak

Centre for Human Structural and Functional Research, Institute of Physical Culture Sciences, University of Szczecin, 17C Narutowicza St., 70-240 Szczecin, Poland

Physical effort is a known factor influencing organism at numerous levels, including maintaining balance in post-exercise secretion of pro- and anti-inflammatory cytokines. It becomes the source of development of athletes' immune response. The direction and intensity of the immune response are determined by T lymphocytes. Thus, the aim of the research was to assess the degree of physical exercise-induced DNA damage and the accompanying cell death of T lymphocytes isolated from peripheral blood of soccer players.

Three groups of soccer players. 50 athletes each, performed progressive YO-YO intermittent recovery test level 1, maximal multistage 20m shuttle run test and a progressive test on a mechanical treadmill, respectively. The concentrations of IL-2, IL-4, IL-6, IL-10, IL-17A, TNF-α and IFN-γ in blood plasma, leukocytes and lymphocytes counts in peripheral blood, and percentage distribution of selected T cell subsets were assessed. Also, T lymphocytes' DNA damage, induction and execution of apoptosis and the assessment of mitochondrial membrane potential, activity of caspases: -8, -9 and -3, and presence of the poly(ADP-ribose) polymerase degradation product was investigated. All the analyses were performed before and after the progressive test and after 17 hours post the physical exercise (at the end of restitution time, to evaluate the long-term biological effect).

The secretion of cytokines related with activated forms of T lymphocyte subsets (Th1, Th2 and Th17), reduction in the total T-cell pool and Th subset distributions and an increase in the percentage of Tc-lymphocytes was observed. Additionally, the long-term biological effect was a significant increase in the percentage of naïve T-cells. DNA damage and induction and execution of cell death was also found.

Differences in the formation of pro-inflammatory response depending on the progressive test protocol used in the study were noticed. Taking the long-term biological response into account, it seems that while the YO-YO IR1 and the test on the mechanical treadmill predominantly induce a rejuvenating effect on the peripheral circulating lymphocyte pool, in the Beep test, molecular mechanisms indicate a more pro-inflammatory systemic response.

The study was partially supported by National Science Centre (grant no DEC-2017/01/X/NZ7/01389).

Extract from *Coriolus versicolor* fungus induces IL-6 and TNF-α production by RAW 264.7 macrophages via phosphatidylinositol 3-kinase signalling pathway

Dagmara Waga[1]*, Kinga Cybulska[1], Tomasz Jędrzejewsk[1], Sylwia Wrotek[1]

1 – Department of Immunology, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University, Lwowska 1 Street, 87-100 Torun, Poland
* email: dagmara97.waga@gmail.com

The extract from *Coriolus versicolor* (CV) fungus is one of the preparations of natural origin that belongs to biological response modifiers. Therefore, it is often used in the treatment of cancer and diseases associated with reduced immunity. The immunostimulatory activities of the CV extract, among others, include the production of cytokines, such as tumour necrosis factor (TNF) α and interleukin-6 (IL-6), which is mediated through by TLR2 or TLR4 signalling pathways. Phosphatidylinositol 3-kinase (PI3K) family is involved in a variety of different cell responses, such as cell survival, cell proliferation and pro-inflammatory cytokine expression. PI3K are activated after stimulation of TLR2, TLR3, TLR4, TLR5, TLR 9 by their ligands.

The aim of the study was to investigate the role of PI3K in the production of IL-6 and TNF-α by RAW 264.7 macrophages stimulated with the CV extract. This effect was estimated using pharmacological PI3K inhibitor LY294002. The cells were co-stimulated with the CV extract (50, 100 and 200 μg/mL) and LY294002 (2 μM) for 24 h or treated with the CV extract alone. Control cells were co-treated with LPS (100 ng/mL) and LY294002 for 24 h or stimulated only with LPS. The level of cell viability and cellular cytotoxicity during stimulation were evaluated using MTT assay and LDH method, respectively. ROS level in macrophages were determined by the DCF-DA method. The amount of IL-6 and TNF-α in culture media were evaluated using standard ELISA methods.

The results showed that the CV extract slightly decreases the viability of RAW 264.7 cells and has not any cytotoxic effect on these cells. Furthermore, it does not change significantly intracellular ROS generation in the cells. On the other hand, the CV extract effectively stimulates IL-6 and TNF-α production in a dose-dependent manner. However, it is a weaker stimulator of expression of genes encoding pro-inflammatory cytokines than LPS. PI3K inhibitor significantly inhibits IL-6 and TNF-α production by macrophages stimulated with the CV extract or LPS.

In the present study we have shown first time that immunomodulatory properties of the CV extract associated with the pro-inflammatory cytokine production are mediated by PI3K signalling pathway.

This research was funded by the POWER project (Universitas Copernicana Thoruniensis In Futuro - modernization of the Nicolaus Copernicus University as part of the Integrated University Program; project no. POWR.03.05.00-00-Z302 /17)

17. Immunopathology

A three-tier approach to diagnosing autoimmune blistering diseases based on analyzing clinical data and results of imaging and biochemical-molecular examinations

Marian Dmochowski

Autoimmune Blistering Dermatoses Section, Department of Dermatology, Poznan University of Medical Sciences, Poznań, Poland

Mucocutaneous autoimmune blistering diseases (ABDs) are requiring precise diagnosis as generally aggressive, having potentially lethal side-effects, treatment is necessary. Nowadays, they can be reliably diagnosed, in addition to the meticulous clinical evaluation including identification of their comorbidities/triggers/sustainers, using a combination approach with biochemical-molecular and imaging laboratory techniques. Taking into account cost effectiveness, I am using an imaging, single-step direct immunofluorescence (DIF) of perilesional tissue and/or of plucked scalp hair for evaluation of IgA, IgG, IgM, C3 as well as IgG4 and IgG1 subclasses deposits visualized with up to three various microscopic systems with an analysis of pattern of deposits. In addition to DIF, still golden standard for diagnosing ABDs, serum studies using biochemical-molecular techniques, namely ELISA, are necessary. Currently, I am using a multi-analyte ELISA enabling the detection of IgG antibodies to desmoglein 1/3, BP180/BP230, envoplakin and type VII collagen in a single procedure. In case of clinical suspicion of dermatitis herpetiformis an ELISA for serum IgA antibodies to tissue transglutaminase, instead of multi-analyte ELISA, is used in addition to DIF. Such a dual imaging/biochemical-molecular laboratory approach is usually sufficient to detect autoimmune nature of a blistering dermatosis in question enabling one to resign from performing H+E histology and indirect immunofluorescence (IIF) studies. Only in cases where it is absolutely necessary, the IIF on a mosaic substrate and/or cells transfected with various epitopes of laminin 332 can be used. Despite substantial progress in diagnosing ABDs heading toward all-in-one methodology encompassing both IgG and IgA autoimmunity and utilizing biomarkers as specific for ABDs as possible, treatment possibilities available to patients are still inadequate as bureaucratic regulations tend to be too restrictive. The aim of future therapeutic efforts should be based on a personalized medicine principle using biotechnology achievements.

The role of pattern recognition receptors in pathology of liver

Jan Żeromski

Chair of Pathomorphology and Clinical Immunology, Karol Marcinkowski University of Medical Sciences, Poznan, Poland

Pattern recognition receptors (PRRs) are crucial portion of the immune system, primarily of innate immunity. Their distribution is almost universal, being expressed on cell surface and intracellularly as well in predominant number of cells of *Eukaryota*. They act as the sentinels of potential risk for a body, including various pathogens but also any noxious compounds, even of self origin. Moreover, PRRs function as signal transducers between innate and adaptive immunity. The liver, large multifunctional organ of the body, exposed to myriads of strange compounds, having various immunogenicity and toxicity, is equipped with a large array of PRRs. In liver disorders they fulfil either role, both, positive and negative. In viral hepatitis C, RIG-1 and TLR3 sense the RNA virus, what results in protective type I interferon production and proinflammatory status. On the other hand, homozygous TLR genotype association appears to be protective in chronic HCV infection. Hepatitis B DNA virus is apparently "invisible" to PRRs, but it was recently denied, in the evidence of their activation in the course of HBV infection. In liver injury and subsequent hepatic fibrogenesis several TLRs, following contact with gut microbiome components induce activation of hepatic stellate cells (HSCs). The latter initiate production of fibrillary collagens leading to liver fibrosis and later cirrhosis. PRRs in persistent chronic liver inflammation are considered pivotal agents in liver carcinogenesis. There are reports that various DAMPs (damage associated molecular patterns) such as free fatty acids, high mobility group B1, in concert with TLRs, contribute to hepatocarcinogenesis.

Cytokines and chemokines in chronic venous insufficiency

Ewa Grudzińska, Zenon Czuba

Department of Microbiology and Immunology, School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia

Chronic venous disease (CVD) affects up to 51% of women and 38% of men in Poland. Pathogenesis of this illness remains unclear. Inflammatory changes are postulated to be an important element of CVD, however, there is very few data on the role of cytokines and chemokines in this disease.

In a series of experiments we assessed the concentrations of 23 cytokines and chemokines produced by lymphocytes (IL-1β, IL-1rα, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12(p70), IL-17A, IFN-γ, RANTES, TNF-α, eotaxin, MIP-1A, MIP-1B, IP-10, MCP-1, G-CSF, GM-CSF, FGF, PDGF-BB and VEGF) in a CVD group in the insufficient great saphenous vein (GSV) and in the healthy cubital vein. The concentrations were also assessed in the cubital vein of a healthy group. We also assessed the effect of PHA stimulation on these concentrations.

The experiments showed significant differences between healthy and insufficient veins, suggesting that the turbulent flow in the insufficient GSV causes proinflammatory changes in the lymphocyte cytokine profile.

Influence of biologically active SP-A protein on lung cancer cell lines

Małgorzata Łagiedo-Żelazowska[1], Mariusz Kaczmarek[2], Jan Sikora[1]

1 – Department of Immunology, Chair of Pathomorphology and Clinical Immunology, Poznan University of Medical Sciences
2 – Department of Cancer Immunology, Chair of Medical Biotechnology, Poznan University of Medical Sciences

Background: The aim of the study was to assess the effect of biologically active SP-A protein on a lung cancer cell lines.

Methods: Three cell lines were examined – two cancerous (squamous cell lung cancer) and one non-cancerous (pulmonary fibroblasts). The experiments were performed using biologically active SP-A protein, which was isolated from pleural effusions, previously collected by thoracocentesis from 34 patients hospitalized in the Department of Pulmonology, Allergology and Pulmonological Oncology of the Poznań University of Medical Sciences and the Greater Poland Pulmonology and Thoracic Surgery Center in Poznań. None of the patients with cancerous pleural effusion have undergone cancer therapy. Using flow cytometry, were analyzed cell cycles of the tested lines, previously cultured *in vitro* in the presence of SP-A. Also, using flow cytometry the expression of TLR2, TLR3 and TLR4 receptors were measured. Finally, RNA from A549 cell line was isolated, in which using the qRT-PCR technique, the expression of selected genes encoding proteins involved in carcinogenesis were measured.

Results: As a result, high SP-A protein concentration in experiments, leads to increase of dead cells of cancer lines, without affecting mortality of the control line. It was found that increasing the level of SP-A protein in the cultures, induced TLR4 expression on the surface of tumor lines. During the experiments, a relationship between high SP-A concentration and increased expression of coding genes for IL-6 was also observed.

Conclusion: Indicated relationship between SP-A proteins and immune responses is significant for the development and course of non-small cell lung cancer, and may also be clinically significant for breaking the tumor suppression. SP-A acts directly on cancer cells and indirectly by activating the TLR4 receptor. The obtained data is an important supplementary data to the current state of knowledge and allows the better understand of the lung cancer carcinogenesis mechanisms. These results may have a significant impact on the development of cancer immunotherapy in which the SP-A protein can be used as a therapeutic agent.

Aberrant BTLA expression in CLL: epigenetic regulation and impact on CLL cells proliferation and ability to IL-4 production

Lidia Karabon[1,2], Anna Andrzejczak[1], Lidia Ciszak[1], Anna Tomkiewicz[1], Aleksandra Szteblich[1], Agnieszka-Bojarska-Junak[3], Jacek Roliński[3], Dariusz Wolowiec[4], Tomasz Wróbel[4], Agata Kosmaczewska[1]

1 – Department of Experimental Therapy, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland
2 – Department and Clinic of Urology and Oncologic Urology, Wroclaw Medical University, Wroclaw, Poland
3 – Department of Clinical Immunology and Immunotherapy, Medical University of Lublin, Lublin, Poland
4 – Department and Clinic of Haematology, Blood Neoplasms, and Bone Marrow Transplantation, Wroclaw Medical University, Wroclaw, Poland

In our previous work, we observed elevated level of BTLA gene transcripts in peripheral blood (PB) B and T cells in chronic lymphocytic leukemia (CLL) patients. Remarkable, despite high BTLA mRNA level, lower BTLA protein expression levels were found on PB CLL cells compared to PB B cells in controls. We hypothesize that aberrant BTLA expression in CLL cells results from epigenetic modifications. The aim of our study was to explore potential miRNAs responsible for the regulation of BTLA expression and reveal the influence of aberrant BTLA expression on cell proliferation and IL-4 production in CLL cells.

Based on the literature overview and *in silico* analysis miR-155-5p was selected as a potential negative modulator of BTLA expression. We observed that the miR-155-5p level is 5 times higher in CLL PB cells compared to normal PB B cells. To evaluate the miR-155-5p function, we transfected PBMCs from 20 CLL patients and 15 controls with miR-155-5p inhibitor or negative control (scrambled miR). After 24 h incubation miR-155-5p/BTLA mRNA and BTLA protein expression levels were measured by qRT-PCR and FACs, respectively.

We found out that after transfection with inhibitor miR-155-5p expression was decreased 10 times either in CLL and control group. Transfection did not affect BTLA mRNA level in both groups. MiR-155-5p inhibition increased significantly BTLA protein level (mean fluorescence intensity) in CLL cells compared to CLL cells transfected with negative control. However, we did not observe this effect both for CLL and normal T cells.

Functional analysis of impaired BTLA expression in CLL PBMC cells performed in cytometric assay showed no differences in fequency of BTLA+IL-4+, BTLA-IL-4+, BTLA+ki67+ and BTLA-ki67+ B cells between CLL patients and controls. Similarly, IL-4 and ki67 fluorescence intensity in BTLA- B cells was also comparable in both groups. In turn, expression level of IL-4 and ki67 in BTLA+ B cells from CLL was found significantly lower compared to corresponding healthy cells. For T cells such differences were not observed in both groups.

Our results indicate that lower expression of BTLA on CLL B cells may be a result of epigenetic regulation by miR155-5p. Inhibition of miR155-5p in CLL B cells appears to be a favorable strategy in CLL by increasing frequency of BTLA-expressing B cells, what, in consequence, might protect from inappropriate proliferation of B cells and secretion of IL-4, which is a growth factor for leukemic cells.

Cytokine expression in patients with obstructive sleep apnea – correlation with susceptibility and clinical parameters of the disease

Chaszczewska-Markowska Monika[1], Gębura Katarzyna[1], Kosacka Monika[2], Brzecka Anna[2], Bogunia-Kubik Katarzyna[1]

1 – Laboratory of Clinical Immunogenetics and Pharmacogenetics, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland.
2 – Clinic of Pulmonology and Lung Cancer, Wroclaw Medical University, Wroclaw, Poland.

Obstructive sleep apnea (OSA) is a major public health problem, that affects a significant portion of the population. It is caused by complete or partial upper airway obstruction that occurs during sleep. OSA may be complicated by many serious diseases, such as cardiovascular diseases, increased incidence of stroke and heart attack, diabetes and metabolism syndrome. Pathological mechanism of OSA remains unclear and this fact prompted us to search for new, dependable and easier to measure biological indicators of OSA susceptibility and severity, as well as disease progression.

We performed simultaneous detection of 45 cytokines in serum of 61 obstructive sleep apnea patients and 16 healthy controls using Human XL Cyt Disc Premixed Mag Luminex Perf Assay Kit (Ref. FCSTM18) by R&D Systems.

Thirty three cytokines were identified as related to OSA susceptibility at $P<0.05$. Twenty four cytokines correlated with OSA susceptibility at $P<0.001$ and levels of all of those were significantly lower in OSA patients when compared to healthy controls, e.g. EGF and CSF3. Levels of many of the cytokines studied were found to be related with various OSA characteristics and clinical parameters, such as forced vital capacity (e.g. TGF-alpha P=0.003; IL6 P=0.001), forced expiratory volume in 1 second (FEV1; e.g. TNF-alpha P=0.027; IL6 P=0.003), desaturation index (DI; e.g. TNF-alpha P=0.023), partial pressure of oxygen (pO2; e.g. TNFSF10 P=0.002) or carbon dioxide (pCO2; e.g. PDGFB P=0.007) and body mass index (BMI; e.g. IL1RN P=0.002; IFN-gamma P=0.025). Levels of many cytokines corresponded with OSA complications, such as stroke incidence (e.g. IL1-beta $P<0.001$; TGF-alpha $P<0.001$), diabetes (e.g. TNFSF10 P=0.002), hypertension (e.g. IL1-beta P=0.007) or chronic obstructive pulmonary disease (e.g. IL10 P=0.001).

Summarizing, our results may greatly improve our understanding of mechanism of OSA development and factors responsible for disease susceptibility.

Do selected SNPs modify the transcriptional activity of the UCP1 gene?

Witkowicz A.[1*], Pravednikova A.E.[2,3*], Kotowska M.[1], Bielawska-Pohl A.[1], Jasek M[1], Issad T[4], Shidlovskii Y.V.[2,3] and Karabon L.[1]

1 - Department of Experimental Therapy, L. Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Weigl 12 Str, 53-114 Wroclaw, Poland
2 – Institute of Gene Biology, Russian Academy of Sciences, 34/5 Vavilov St., Moscow, 119334 Russia
3 – I.M. Sechenov First Moscow State Medical University, 8, bldg. 2 Trubetskaya St., Moscow, 119048 Russia
4 – Institut Cochin, Department of Endocrinology , Metabolism and Diabetes, 22 Rue Mechain, 75014 Paris, France.
* authors contribute equally to this work

In the past decade, there has been a dramatic increase in chronic diseases like diabetes, hypertension, and obesity, the conditions which lead to the development of cardiometabolic diseases (CMDs). Various studies have shown that alterations in number or density of mitochondria and their oxidative mechanism are associated with development and progression of CMD. Uncoupling protein 1 (UCP1) located in the mitochondrial inner membrane, mainly expressed in brown adipose tissue (BAT) is a key factor of thermogenesis and energy expenditure. We hypothesize that functional single nucleotide polymorphisms (SNPs) of *UCP1* gene might altered expression and activity of UCP1 and might contribute to the predisposition to CMDs.The aim of our study was to determine, whether 5' variations: A-112C (rs10011540) and A-3826G (rs1800592) have functional significance in modulating promoter activity of the *UCP1* gene.

The promoter activity of *UCP1* gene was studied using the Dual-Glo Luciferase Assay. Wild-type (A-112C A variant) and mutant (A-112C C variant) 1077 base pairs (bp) proximal promoter sequences were cloned upstream of the firefly luciferase reporter gene in the pGL3-Basic vector. Then 523 bp putative enhancer bearing variants of A-3826G SNP (A or G) or control sequence were cloned downstream to these vectors. Finally, the following vectors were obtained: A-112C A; A-112C C; A-112C A/A-3826G A; A-112C A/A-3826G G; A-112C C/A-3826G A; A-112C C/A-3826G G, and A-112C A/control-enh. The respective vectors were co-transfected with the Renilla luciferase pGL4.74 reporter vector using FugeneHD into PAZ6 cell line, differentiated into BAT. Luciferases activity was measured 48 h after transfection.

No difference was found in the promoter activity for A-112C SNP variants in PAZ6 cells line. However lower luciferase activity for A-3826G SNP mutant G variant in PAZ6 cells either in normal and in simulating (retinoic acid and norepinephrine) condition were found while the differences did not reach statistical significance. The obtained results needs to be confirmed in future study.

Our results indicated that *UCP1*A-112C SNP is not involved in the transcription regulation of UCP1 expression, while the A-3826G SNP might modulate transcriptional activity of *UCP1* gene.

Project supported by: U-GENE FP7-PEOPLE-2012 IRSES-319010 and Polish Ministry of Science and Higher Education (W15/7.PR/2016); we kindly thanks Dr Issad (Institute Cochin, France), for providing PAZ6 cell line.

18. Innate Immunity in Protection of Barrier and Internal Organs

Overview: Neutrophils as sentinel cells of the immune system

Janusz Marcinkiewicz

Department of Immunology, Jagiellonian University Medical College, Krakow, Poland

The general aim of the lecture is presentation of the current opinions on the dual role of neutrophils in acute and chronic inflammation (*Neutrophils – friends or foes in immunity?*). The following aspects of the research of neutrophils will be discussed:

- Neutrophils – sentinel cells of innate immunity
- Various outcomes of the neutrophil response to infections – a tug of war between neutrophils and microbes (planktonic bacteria *vs* bacterial biofilm)
- *Hypothesis:* Biofilm microenvironment and biofilm-associated neutrophils (BANs)
- Beneficial role of neutrophils in acute inflammation
- Detrimental role of neutrophils in chronic inflammation
- Fate (types of deaths) of neutrophils at a site of inflammation
- Neutrophils in pathogenesis of viral infections (RSV, COVID-19)
- SARS-CoV-2 *vs* neutrophils, NET formation and cytokine storm.

Interplay between neutrophils, their NETs, and platelets in the liver: consequences for the outcome of systemic inflammation and "obesity paradox in sepsis"

Elzbieta Kolaczkowska

Laboratory of Experimental Hematology, Institute of Zoology and Biomedical Research, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland

Systemic inflammation remains a life-threatening organ dysfunction caused by a dysregulated host response to infection and is associated with high mortality. Some of the major immunological events deciding about the outcome of sepsis occur in the liver, a unique organ and frontline immune tissue. The organ contains the largest collection of phagocytes in the body and is able to mount a rapid and robust immune response. Dynamic interactions between the numerous populations of immune cells in the liver (Kupffer cells, tissue specific macrophages; neutrophils; iNKT cells; and also platelets) are key to overall health status. The recent years brought up discoveries that changed our perception of platelets as simple bodies participating in coagulation. In fact, nowadays platelets are accepted by immunologists as "functional immune bodies" that actively initiate and/or participate in immunological processes. Many of the discoveries on this topic came from studies utilizing intravital microscopy. With this approach direct interactions of cells can be followed in real time in vasculature of live mice. We showed previously that Kupffer cell-neutrophil-platelet interactions are critical for initiation and the course of sepsis, and they might lead to formation of neutrophil extracellular traps (NETs) by neutrophils. Furthermore, platelets are critical for the course of sepsis in the context of immunometabolic state of the organism. In obese mice less platelets are detected in liver sinusoids during endotoxemia, and they have fewer interactions with neutrophils. Moreover, independently of the cause of obesity, high fat diet or lack of leptin receptor (ob/ob mice), less NETs are formed by septic neutrophils present in the liver vasculature. This is not intrinsic in nature as NET formation is unchanged by neutrophils isolated from such animals and studied *ex vivo*. Importantly, upon selective platelet transfer from lean to obese mice, the NET formation can be at least partially restored. As such, the diminished platelet-neutrophil interactions might thus explain the "obesity paradox in sepsis" according to which obese individuals might be protected from sepsis-related mortality. All the above platelet-neutrophil-NET interactions and interdependencies are critical for the course of sepsis and thus survival during this systemic inflammation. Therefore, a pharmacological intervention at these specific targets – alone or together – might provide new therapeutic tools.

Neutrophils in pathogenesis of skin disease-psoriasis

Joanna Cichy

Dept. of Immunology, Faculty of Biochemistry, Biophysics & Biotechnology, Jagiellonian University

Here we discuss some of the key pathways of neutrophil engagement in chronic inflammatory skin disorder-psoriasis. Skin-infiltrating neutrophils are the hallmark of psoriasis, yet a role of neutrophils in pathophysiology of this condition remains elusive. Neutrophils are broadly classified into conventional neutrophils (PMNs) and low density granulocytes (LDGs). Expansion of neutrophils phenotypically resembling LDGs was observed in several solid tumors and chronic inflammatory diseases. In tumors, LDG-like cells are equipped with immune suppressive properties, whereas in chronic inflammatory diseases LDGs are proposed to be mostly proinflammatory. LDGs are better than PMNs in the generation of NETs, which may contribute to the pathology of psoriasis. We hypothesized that LDGs and PMNs differ in levels of unrestrained neutrophil elastase (NE) that supports NET generation. Here, we show that individuals with psoriasis contain elevated levels of LDGs and that in contrast to PMNs, LDGs display higher staining for NE and lower staining for its inhibitor SLPI. Distinctive staining for NE and SLPI in LDGs and PMNs did not result from differences in their protein levels but likely dependent on different subcellular sequestration of these proteins. The heterogeneity between blood-derived LDGs and PMNs was somewhat reminiscent of the differences in NE and SLPI staining patterns observed in psoriasis skin-infiltrating neutrophils. The distinct profile of NE and SLPI in LDGs and PMNs coincided with altered migratory responses of these cells to cutaneous chemoattractants. Collectively, differential NE and SLPI staining identifies common attributes of both circulating and skin-infiltrating neutrophils, which may guide neutrophil migration to distinct skin regions and determine the localization of LDGs-mediated cutaneous pathology.

Acknowledgments: This work was supported by grants from Polish National Science Center UMO-2014/12/W/NZ6/00454 and UMO-2017/25/B/NZ6/01003.

Clearance of extracellular proteins from liver vasculature during resolution of systemic inflammation visualized by intravital microscopy

Michal Santocki[1], Elzbieta Kolaczkowska [1]

1 – Laboratory of Experimental Hematology, Institute of Zoology and Biomedical Research, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland

Background: Sepsis is a life-threatening systemic inflammatory condition that leads to complex immune responses. It is characterized by influx of neutrophils, the foot soldiers of innate immunity, into highly vascularized organs, such as the liver. Once infiltrating the organ, the cells release various mediators, including antibacterial proteins and enzymes, of which some are deposited on the endothelium. The systemic over-concentration of the mediators might cause long term side effects leading to endothelium and/or organ damage if inappropriately or untimely removed. Therefore, the aim of this study was to identify mechanisms by which the cells present in the liver remove extracellular proteins by application of intravital (*in vivo*) microscopy that allows to observe dynamic processes in living animals in real time.

Methods: Following the resolution of ipopolysaccharide-induced sepsis in C57BL/6J mice, we verified the kinetics of removal of extracellular proteins deposited alongside vasculature during the initial immune response. Applying spinning-disk confocal microscopy we focused on the engulfment of neutrophil elastase (NE) by immune cells in the sinusoids of the inflamed liver. After performing a series of optical scans (*z-stacks*), we reconstructed the 3D structure of the liver and the cells therein present, with the use of advanced image analysis software (IMARIS). Next, we measured the volume of engulfed NE by the immune cells, using MeasurementPRO module for IMARIS software.

Results and conclusions: Using appropriate isotype controls and Fc receptor blocking antibodies, we successfully verified the specificity of our approach. Next, we identified the cells (phagocytes) involved in NE engulfment and measured the amount of intracellular NE (MeasurementPRO). With the use of specific monoclonal antibodies we established that macrophages of the liver (Kupffer cells) and neutrophils infiltrating the organ are able to engulf neutrophil elastase. Kupffer cells internalized more NE compared to neutrophils and the process of engulfment turned out to be elastase-specific, not antibody-driven, as proven by the use of appropriate control antibodies. Ultimately, this novel approach allowed us to create foundations for future studies on removal of various molecules/structures from blood and endothelium.

The study was supported by National Science Centre of Poland, OPUS 15, 2018/29/B/NZ6/00713.

PMNs or LDNs, it is a question. On the way to find the type of neutrophils infiltrating lesional skin in mouse model of psoriasis-like dermatitis

Kwiecińska Patrycja, Dziadowiec Alicja, Skrzeczyńska-Moncznik Joanna and Cichy Joanna

Immunology Department, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University

Recent data indicate a heterogeneity of circulating neutrophils. Classical neutrophils (PMNs) and low-density neutrophils (LDNs) are characterized by expression of the same differentiation markers, but differ in buoyancy, and sediment separately after density gradient centrifugation. Low-density neutrophils are important cells for the pathogenesis of many inflammatory diseases. Our previous data showed that differences in staining for elastase (NE) and its inhibitor SLPI reveal heterogeneity among human neutrophil subpopulations in psoriasis.

We also reported that cells with different NE and SLPI staining pattern might infiltrate distinct lesional skin regions of psoriasis patients. Here we show that in common with individuals suffering from psoriasis, lesional murine skin in experimental model of psoriasis was strongly infiltrated with neutrophils. Likewise, similar to humans, the percentage of circulating LDNs was found to be increased during psoriasis-like dermatitis in mouse. Using adoptive transfer experiments with donor PMNs and LDNs to recipient mice, we observed a tendency for more robust accumulation of LDNs in psoriatic-like skin lesions. However, in contrast to human neutrophils, in vitro chemotaxis experiments did not demonstrate more efficient migration of LDNs to skin extracts compared with PMNs. In addition, NE and SLPI staining pattern of mouse PMNs and LDNs did not recapitulate differences in these neutrophil subsets in humans. Together these data suggest that NE-and SLPI-dependent impact on neutrophil subset biology in mouse and humans, such as migration and accumulation in the chronically inflamed skin might be different.

Evaluation of effector functions of RAW 264.7 macrophages in the presence of extracellular vesicles and lipopolysaccharide – a comparative study

Anna Biała, Weronika Ortmann, Elżbieta Kołaczkowska

Laboratory of Experimental Hematology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Krakow, Poland

Extracellular vesicles (EV) are small membrane-enclosed structures that can be released by any cell type under either *in vivo* or *in vitro* conditions, in hemostasis and pathological processes. EVs are key players in intercellular communication and participate in modulation of the immune response. One of the EVs subtype called microvesicles (MV) contributes to subsequent phases of systemic inflammation aka sepsis. The aim of this study was to determine effects of microvesicles collected from mice with liposaccharide (LPS)-induced sepsis (endotoxemia) on the immunological activity of macrophages, particularly cell viability, adhesion to the substrate, mitochondrial activity, nitric oxide (NO) and reactive oxygen species (ROS) production. MVs were isolated from two different body compartments: blood plasma and exudative/inflammatory fluid from the peritoneal cavity of mice with endotoxemia. Effects of two MV concentrations 80 x 10e5 MV/10e5 cells and 160 x 10e5 MV/10e5 cells were studied. The experiments were carried out on RAW 264.7 murine macrophage-like cell line. The obtained data indicate that irrespective of MV's origin or concentration, they up-regulated macrophage viability but did not influence other parameters. Furthermore, a relationship between NO levels produced by the cells in response to the MV stimulation and a passage number was detected. In particular, less NO was secreted by cells from lower passages than from the higher ones. Moreover, changes in the intensity of NO production were accompanied by alternations in RAW 264.7 macrophage phenotype and functional stability. Overall, our results revealed only a minor impact of MVs applied in studied concentrations on the effector functions of murine macrophages.

The study was supported by National Science Centre of Poland, PRELUDIUM 15, 2018/29/N/NZ6/01073.

Effects of itaconic acid on inflammatory responses of murine macrophages and neutrophils and formation of neutrophil extracellular traps (NETs)

Gabriela Burczyk, Iwona Cichoń, Elżbieta Kołaczkowska

Laboratory of Experimental Hematology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Kraków, Poland

Itaconic acid is an unsaturated, organic dicarboxylic acid produced in macrophages during broken Krebs cycle. Its biosynthesis is catalyzed by cis-aconitic acid decarboxylase (CAD)/immune-responsive gene 1 protein (IRG1). Itaconic acid has bactericidal and anti-inflammatory properties associated with metabolic-immune interactions. Immunometabolism focuses on changes that occur in intracellular metabolic pathways in immune cells during their activation. Thus by modulation of cellular metabolic parameters, functioning of leukocytes can be changed providing a potential therapeutic value. Herein we aimed to verify if the Krebs cycle metabolite affects inflammatory response of murine macrophage cell line RAW 264.7 when applied exogenously, and to determine if it also affects neutrophil activity, and in particular their ability to form neutrophil extracellular traps (NETs). NETs were originally thought to be beneficial to host defense through their ability to trap and immobilize pathogens, but later studies have shown that they can also contribute to organ and tissue damage, e.g. during sepsis. In the studies, murine macrophages (RAW 264.7) and neutrophils (bone marrow-derived) were treated with various concentrations of 4-octyl itaconate (4-OI) in the presence or absence of lipopolysaccharide (LPS). At first, the viability/cytotoxicity tests were performed (NBT, MTT, CV, PrestoBlue) to verify the compound safety. Then, to elucidate the role of Krebs cycle metabolite in the cell inflammatory functions, nitric oxide levels along with expression of inducible nitric oxide synthase (iNOS) in macrophages was evaluated, as well as NET release by neutrophils was determined. In the presence of 4-OI, macrophages produced less NO upon LPS stimulation and accordingly, abolished iNOS expression was observed, in comparison to macrophages threated with LPS only. In neutrophils, the confocal microscopic analysis clearly showed that formation of NETs by LPS stimulated cells pre-treated with 4-octyl itaconate was diminished. We conclude that itaconate has an immunosuppressive impact, both on macrophages and neutrophils, as pre-exposure of the cells to this metabolite downregulates their cellular functions and overall confirms immunometabolic regulation of studied processes.

The study was supported by National Science Centre of Poland, OPUS 15, 2018/29/B/NZ6/00713.

Metabolic requirements for neutrophil extracellular trap (NET) formation in obesity during systemic inflammation

Iwona Cichon[1], Weronika Ortmann[1], Elzbieta Kolaczkowska[1]

1 – Laboratory of Experimental Hematology, Institute of Zoology and Biomedical Research, Jagiellonian University, Krakow, Poland

Chronic, low grade inflammation is a hallmark of obesity and altered course of systemic inflammation/sepsis was suggested in obese individuals. One of the highlights of the onset of sepsis is infiltration of neutrophils and the formation of neutrophil extracellular traps (NETs). These structures are formed by highly activated neutrophils and consist of decondensed DNA to which granular (e.g. neutrophil elastase, NE) and nuclear (e.g. histones) proteins are attached, and their main function is to capture and eliminate pathogens. Thus far, we observed that obese mice release much less NETs during endotoxemia than lean individuals *in vivo*. The mechanism behind observed phenomenon is still elusive and some immunometabolic regulations were proposed.

Hence, the aim of this study was to determine metabolic requirements in neutrophils from obese and lean mice, both in physiological (healthy mice) and inflammatory conditions (lipopolysaccharide (LPS)-induced endotoxemia) for NET formation following *ex vivo* LPS stimulation. High fat diet obesity (C57BL/6J) was established and endotoxemia was induced by intraperitoneal injection of lipopolysaccharide. Neutrophils were collected from the bone marrow of lean (ND, normal diet) and obese (HFD, high fat diet) mice (healthy and endotoxemic) and cellular metabolism was studied with Seahorse analyzer and applying a set of metabolic inhibitors. NET formation was verified by spectrofluorometric analysis (extracellular DNA) and fluorescence/confocal microscopy (extDNA and nuclear/granular proteins).

Obtain results revealed that glucose and glycolysis along with pentose phosphate pathway (PPP) are involved in NETs release by neutrophils from lean mice. Whereas neutrophils from healthy obese individuals utilize those routes in physiological conditions and additionally display high flexibility to switch metabolism toward fatty acid oxidation, however during sepsis, after second hit of LPS (*ex vivo*) neutrophils from obese mice exhibit "exhaustion" and hardly release NETs. Additionally, we noted increased NETs ejection after ATP synthase blockage in neutrophils from both groups of mice, which may suggest more active glycolysis.

In conclusion, our study revealed that formation of NETs is altered in obese subjects and it depends on metabolic changes related to obesity.

The study was supported by National Science Centre of Poland, OPUS 15, 2018/29/B/NZ6/00713.

Formation of macrophage extracellular traps (METs) by bone marrow-derived macrophages (BMDMs) is reactive nitrogen species (RNS)-dependent

Dominika Drab[1], Michal Santocki[1], Elzbieta Kolaczkowska[1]

1 – Laboratory of Experimental Hematology, Institute of Zoology and Biomedical Research, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland

Extracellular trap (ET) formation is widely studied in neutrophils, although also other innate immune cells, such as macrophages, are able to release the extracellular DNA decorated with nuclear and granular proteins. Macrophage extracellular traps (METs) were shown to serve as defense weapons against the pathogens. Mechanisms involved in their formation are still fragmentary, hence, we aimed to study mechanism of their formation by bone marrow-derived macrophages (BMDMs) upon exposure to various immunostimulants and compounds, including lipopolysaccharide (LPS) and zymosan. In particular, we focused on involvement of reactive nitrogen species (RNS) in MET formation. Additionally, we aimed to verify if modification of the established protocol, by freezing bone marrow cells prior to their differentiation, will affect MET release. To obtain BMDMs, bone marrow cells were frozen in liquid nitrogen shortly after isolation and kept in these conditions until differentiation. In other studies, freshly isolated bone marrow was used. Conditioned medium, containing macrophage colony-stimulating factor (M-CSF), was derived from the mouse fibroblast L929 cell line cultured for 10 days. Subsequently bone marrow cells, fresh and thawed, were cultured in the conditioned medium for 7 days till they differentiated into macrophages ($F4/80^+$, $CD11a^+$, flow cytometry). Then BMDMs were stimulated with LPS and/or zymosan to induce METs. Some cells were pretreated with nitric oxide synthase (NOS) inhibitors (L-NAME, 1400W) or nitric oxide donor (SNAP) or peroxynitrite. After overnight stimulation METs were visualized by fluorescence/confocal microscopy upon immunocytochemical staining detecting MET proteins, histones (H2A.X) and MMP-9 attached to extracellular DNA. Fully differentiated and functional macrophages (adherent, metabolically active, $iNOS^+$, NO producing) suitable for MET studies were obtained both, from fresh and cryopreserved bone marrow cells. Upon stimulation, both BMDMs were able to produce METs. As NOS inhibitors (L-NAME, 1400W) halted the MET formation by BMDMs upon stimulation with LPS, and SNAP and peroxynitrite induced MET release, we postulate that formation of the traps by macrophages depends on RNS.

The study was supported by National Science Centre of Poland, OPUS 15, 2018/29/B/NZ6/00713.

Filaggrin-insufficient keratinocytes produce exosomes characterized by reduced capacity to promote CD1a-dependent responses

Kobiela A.[1], Hewelt-Belka W.[2], Lewandowska A.[1], Frackowiak J.[1], Hovhannisyan L.[1], Biernacka A.[1], Michalska M.[1], Czechowska E.[1], Wang X.[3, 4], Giannoulatou E.[5], Hester J.[6], Ogg G. S.[3], Gutowska-Owsiak D[1,3].

1 – University of Gdansk, Intercollegiate Faculty of Biotechnology UG & MUG, Poland,
2 – Department of Analytical Chemistry, Chemical Faculty, Gdansk University of Technology, Gdansk, Poland,
3 – MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford,
4 – State Key Laboratory of Military Stomatology, Department of Oral Medicine, School of Stomatology, The Fourth Military Medical University, Xi'an Shaanxi Province, China,
5 – Computational Biology Research Group, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford,
6 – Transplantation Research Immunology Group, Nuffield Department of Surgical Sciences, University of Oxford, Oxford

Background: Filaggrin (FLG) is a key structural protein expressed in epidermal keratinocytes. Mutations in the filaggrin gene (*FLG*) are strongly linked to atopic dermatitis (AD) and allergic inflammation occurring later in life at distant body locations in AD patients. Keratinocytes secrete exosomes, small, lipid-rich membrane vesicles to communicate with distant cells, including within the immune system. Here, in a sh knock down model we investigated if filaggrin insufficiency impacts the ability of keratinocyte-derived exosomes (KC_{exo}) to influence antigen-specific T cell responses.

Methods: KC_{exo} were isolated from conditioned media by ultracentrifugation. T cell responses to peptides, whole protein and lipid neoantigens generated by phospholipase A2 (PLA2) were measured by IFNγ ELISpot and ELISA upon the addition of exosomes. Lipidome and proteome analysis of keratinocytes and KC_{exo} was performed by mass spectrometry.

Results: Reduced capacity of $shFLG_{exo}$ to mediate CD1a-dependent T cell responses was observed; T cell responses to peptides or whole protein were not affected. Lipidomic analysis of shFLG cells suggested extensive metabolic reprogramming in lipid pathways in comparison to the shC cells. The analysis of the exosomal data revealed decreased abundance of long chain polyunsaturated fatty acids (LC-PUFAs) and predominance of saturated fatty acids (SFAs) in $shFLG_{exo}$ ShFLG cells differed in the expression a number of proteins involved in lipid metabolism, which included downregulation of long-chain-fatty-acid--CoA ligase ACSL3, a key enzyme in multiple lipid pathays.

Conclusions: FLG insufficiency in keratinocytes affects lipid metabolism pathways and contributes to alterations in the lipid-enriched exosomal compartment. Decreased presence of LC-PUFA species in $shFLG_{exo}$ results in a reduced supply of lipids which could act as sources of neoantigens for CD1a binding. This has a negative effect on the ability of the $shFLG_{exo}$ to induce CD1a-specific T cell responses, which could potentially contribute to chronic inflammation in tissues distant form the skin and promote additional allergic manifestations in AD patients.

Lazy neutrophils – how deficiency of DGAT1 disturbs activity of mouse neutrophils

Patrycja Kwiecińska, Kamila Kwiecień, Alicja Uchańska

Department of Immunology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, 30-387 Kraków, Poland

Psoriasis is a chronic immune-mediated skin disorder, characterized by keratinocytes hyperproliferation and a significant inflammatory infiltrate. One of its histological hallmarks is epidermal accumulation of neutrophils. Among all leukocytes, mouse neutrophils have the highest expression of the acyl-CoA:diacylglycerol acyltransferase 1 (DGAT1) gene. DGAT enzymes catalyze the synthesis of triglycerides (TGs), which are the major form of stored energy in mammals. TG is a source of glycerol, which can be used in glycolysis – the main metabolic pathway in neutrophils. Additionally, DGAT1 converts retinoic acid (RA) to retinyl esters. RA is a potent signaling molecule, involved in the regulation of a wide range of physiological processes. RA can stimulate differentiation of the myeloid lineage, and play key role in neutrophil maturation and differentiation. In addition, therapeutic administration of RA exerts anti-inflammatory effects in dermatological diseases, such as psoriasis, at least partially by inhibiting pro-inflammatory activity of neutrophils (inhibition of chemotactic responses, superoxide anion production, and lysosomal enzyme release).

Since DGAT1 regulate the homeostasis of lipids and retinoids, we hypothesized that lack of DGAT1 will lead to inhibition of pro-inflammatory activity of neutrophils and reduction of psoriasis symptoms. To verify this hypothesis, we performed experiments using Aldara™-treated mouse model of psoriasis on mice lacking DGAT1. We observed that skin changes in Dgat1KO mice covers a larger area and were more scaly, but less reddened than in WT mice. Mice lacking DGAT1 show also inhibition of epidermis thickening. It was correlated with significantly lower number of neutrophils infiltrating psoriatic skin of Dgat1KO mice, but no changes in neutrophil number in blood or spleen. To explain the differences in neutrophil infiltrates, we tested their chemotactic abilities using sterile peritonitis and *in vitro* chemotaxis assay. In both assays we observed significantly lower number of neutrophils lacking DGAT1. To examine if DGAT1 may influence other pro-inflammatory activities of neutrophils, we performed oxidative burst test, and we showed that neutrophils from Dgat1KO mice after PMA activation consume even 2-fold less oxygene to produce ROS, then neutrophils form WT mice.

In conclusion, our data suggest that DGAT1 modulate proinflammatory activity of neutrophils. Neutrophils lacking DGAT1, probably due to the reduced availability of TGs and higher level of RA, are less active compared to WT neutrophils.

Microparticle (MP)–neutrophil extracellular trap (NET) crosstalk during systemic inflammation in mice

Weronika Ortmann[1], Iwona Cichon[1], Michal Santocki[1],
Monika Baj-Krzyworzeka[2], Elzbieta Kolaczkowska[1]

1 – Laboratory of Experimental Hematology, Institute of Zoology and Biomedical Research, Jagiellonian University, Krakow, Poland
2 – Department of Clinical Immunology, Jagiellonian University Medical College, Krakow, Poland

Neutrophils are key cells involved in the body's immune responses. They reach the site of inflammation first and immediately start performing their effector functions. Neutrophil are known for their ability to release neutrophil extracellular traps (NETs) as well as microparticles (MPs). NETs, composed of DNA and granular proteins, immobilize pathogens limiting their spread throughout the body whereas MPs are (nano-micrometer) structures containing numerous bioactive molecules, such as proteins, lipids or genetic material. Thus, they are armed to modulate various processes once internalizing by target cells. Importantly, MPs can attach to the NET structure, as well as induce NET ejection. The aim of the study was to investigate secretion of MPs in different body compartments and correlate it with NET release during systemic inflammation (endotoxemia induced by lipopolysaccharide). *In vivo*, MP and NET formation was visualized and evaluated with intravital microscopy (IVM) in liver sinusoids (vasculature) of healthy and endotoxemic/septic mice. MPs were additionally analyzed *ex vivo* by Nanoparticle Tracking Analysis (NTA). The NTA revealed that low amounts of MPs were present in fluids of healthy mice and their numbers did increase over the course of sepsis. The *in vivo* studies (IVM) showed correlation between MP release and NET formation and indicated that numbers of neutrophil MPs increase over time during systemic inflammation positively correlating with NETs as well as numbers of neutrophils infiltrating the liver. Moreover, MPs present in sinusoids co-localized with the released traps. This study strongly indicates a connection and interplay between MPs and NETs during sepsis, and suggests that the two could serves as biomarkers of the systemic inflammation.

This study was funded by the Science Centre of Poland (grant No. 2018/29/N/NZ6/01073).

19. Modern diagnostics of immune-related diseases

Can NETs markers play a role in clinical diagnostics?

Urszula Demkow

Dept. of Laboratory Diagnostics and Clinical Immunology of Developmental Age Medical University of Warsaw

NETs were discovered in 2003 as extracellular traps released by neutrophils, composed of decondensed chromatin and granule proteins. NETs-inducing agents include bacteria, fungi, protozoa, viruses, platelets, cytokines, and nitric oxide donors. Although NET generation has been described initially as an antimicrobial mechanism, recent data suggest that NETs contribute to lung injury, vascular thrombosis, autoimmunity and cancer. Numerous conditions are characterized by the presence of circulating neutrophil extracellular traps (NETs). Circulating surrogate markers of NETs in plasma are complexes of DNA and myeloperoxidase, citrullinated histone H3, cell-free DNA, nucleosomes and neutrophil elastase. Circulating NETs markers correlate with markers of inflammation and endothelial damage in COVID-19 emphasize the relevance of the virus for the vasculature, and centers the causes for patients' demise on the microvascular thrombosis aspect of the infection. Derangement of the endothelial activation/damage marker VWF (von Willebrand factor) and its protease, ADAMTS13, correlated with with markers of NETs. Clinical prognosis is important in guiding interventions and planning future care. Objective tools are therefore needed. Blood biomarkers are appealing due to their rapid measurement and objective nature and their role as prognostic indicator. NETs biomarkers in blood and tissues as determinants of clinical outcome in various conditions will be discussed. For example higher circulating NETs levels are associated with the need for respiratory support and with high mortality in Covid-19 patients mortality, which confirm some studies. Circulating DNase may be another important puzzle piece in various diseases. Dnase1 is naturally regulating the amount of circulating extracellular chromatin, and intact endogenous plasma Dnase activity is essential for homeostasis and survival. about its association with disease severity and potential effects on circulating NETs markers.

Application of Capture Bead Assays in immunodiagnostics

Kazimierz Węglarczyk

Cracow, Poland

In immunological diagnostics, many different platforms have been developed where antibodies are being used for the capture or detection of different factors. Usually, if antibodies are used to capture analytes, a support, such as the wall of a microtiter-plate well, as in ELISA test, is required to create a layer of antibodies enabling capturing. If the purpose of antibodies is to detect the presence of captured analytes, these reporter antibodies can be used directly as conjugates with reporter molecules, such as enzymes or fluorophores, depending on the assay type and the platform used for collecting and analysing data. Recently, the miniaturization of analytical systems is making a great progress and offers interesting alternatives for traditional immunoassays. These include bead-based immunoassays, where the surface of the microspheres/beads is the place on which an immunoassay is performed. Bead-based immunoassays are often used in laboratories dealing with solid organ transplantation. These tests are rapid and efficient for determining human leukocyte antigens (HLA) as well as the anti-HLA antibodies required for checking the compatibility between donors and recipients. The main obstacle in these tests is a high degree of HLA diversity. For this reason, the Luminex platform, able to discriminate and analyse up to 100 individual beads in a single multiplexed assay, has been developed. A unique bead signature is created by filling each bead with two different fluorescent dyes. By adjusting the concentration of the two dyes, a set of 100 identifiable beads was developed. Each could be coated with a different antigen or specific sequence, allowing positive immunoassay results to be automatically correlated to a unique anti-HLA antibody or HLA specificity. In this case, the principle of checking the HLA specificity (HLA typing) involves the hybridization of reverse sequence-specific oligonucleotide probes, which are attached to a unique colour coded microsphere to identify HLA class I and class II alleles. The amplified (PCR) target DNA, where specific, biotinylated primers were used, allows detection with the use of R-Phycoerythrin-conjugated Streptavidin. The PCR product is then denatured and hybridised to complementary DNA sequences conjugated to fluorescently coded beads. In the case of detecting the presence anti-HLA antibody in patient serum, a unique colour coded beads are coated with different HLA antigens. Detection of positive signal occurs by using PE-conjugated specific anti-human antibodies. Another platform, which can be utilized as a bead-based immunoassay is classical Flow Cytometry. Here, Cytometric Bead Array (CBA) can be used, which provides a method of capturing an analyte or set of analytes with beads of known size and fluorescence. Usually, the CBA system is used to detect the presence of soluble factors such as cytokines or chemokines. In such cases, flow cytometry is a commonly used method and the possibility to detect many soluble factors in one sample makes it more efficient and attractive than classical immuno-enzymatic test (ELISA).

IgG4 diagnostic traps

Aleksandra Matyja-Bednarczyk

2nd Department of Internal Medicine, Jagiellonian University Medical College, Krakow, Poland
Department of Outpatient Clinic for the Immunological and Hypercoagulable Diseases, The University Hospital in Kraków, Kraków, Poland

Immunonoglobulin G4-related disease (IgG4-RD) is an immune-mediated fibroinflammatory condition that is capable of affecting multiple organs.

The diagnosis of IgG4-RD is based upon the combination of characteristic histopathologic, serologic, clinical and radiologic findings.

Diagnosis requires tissue biopsy of an affected organ with characteristic histological findings. Serum immunoglobulin G4 is often elevated but this is not always the case.

The major disorders that should be distinguished from IgG4-RD include pancreatic cancer, primary sclerosing cholangitis, cholangiocarcinoma, Sjogren's syndrome, anti-neutrophil cytoplasmic antibody-associated vasculitis, Castelman disease and infectious aortitis.

I will present diagnostic difficulties using the example of patient cases with IgG4-RD.

20. New aspects of innate immunity in cancer

Dual role of neutrophils in cancer

Ewa Jabłońska

Department of Immunology, Medical University of Bialystok, Waszyngtona 15A, 15-269 Bialystok, Poland

Tumor-associated leukocytes play a crucial role in tumor-host interactions, which are responsible for the development of tumor. It is well established that among these cells, tumor-associated neutrophils (TANs) constitute a significant part of the tumor microenvironment. TANs can be modulated by different cytokines and chemokines, secreted by tumor cells and immune cells, and may acquire either anti-tumor or pro-tumor activity.

In recent years, there has been a debate on the dual role of neutrophils in cancer. Numerous studies have contributed to the identification of new mechanisms of anti-tumor cytotoxicity of neutrophils, such as the antibody-dependent cancer cells destruction through trogocytosis, or cytotoxicity by Cathepsin G/ RAGE axis.

On the other hand, new mechanisms of the tumor-promoting activity of those cells have also been identified, including the PD-L1 interaction with PD-1 leading to the suppression of cytotoxic T cells, or the ability of the cells to release pro-tumor TNF superfamily molecules involving APRIL (a proliferation-inducing ligand) and BAFF (B-cell-activating factor).

According to many authors, dual role of neutrophils in cancer is associated with the heterogeneity of those cells in the tumor and the circulation: N1 and N2 phenotypes of TANs in mouse model, APC-like hybrid TANs and canonical TANs in humans, as well as Normal Density Neutrophils (NDNs), Low Density Neutrophils (LDNs) and High Density Neutrophils (HDNs) populations.

Our recent research has been focused on new aspects of the pro-tumor activity of neutrophils including, among others, the population of IL-17^{positive} LDNs, NETs generation or neutrophils-derived BAFF molecule activity.

On the basis the currently available knowledge and the results of our own and other authors' research, it can be assumed that neutrophils promote, rather than suppress, tumor growth. The pro-tumor activity of neutrophils is confirmed by the N/L ratio, the increase of which is correlated with poor clinical outcomes in multiple cancers.

The aforementioned observations have driven the research on the possibility of regulating this disadvantageous aspect of neutrophil activity, which may serve as the reference point for new therapies. Our own observations suggest a beneficial effect of flavonoids in reducing the pro-tumor activity of those cells.

The implication of tumor-associated macrophages in tumor progression and drug resistance

Magdalena Klink

Institute of Medical Biology, Polish Academy of Sciences, Lodz, Poland

The effectiveness of conventional anti-tumor therapies depends on the biological activity of tumor cells**,** as well as on the complex cells' interactions occurring in solid tumor microenvironment (TME). Similarly, the tumor cells progression and metastasis are also determined by behavior of the TME components, particularly immune cells. The key cells of immune system involved in both chemoresistance and progression of disease are tumor associated-macrophages (TAMs). TAMs originate both from tissue resident macrophages and from the circulating monocytes. They are divided into two main groups: anti-tumoral pro-inflammatory M1 type and pro-tumoral immunosuppressive M2 type. Growing evidence has shown that most TAMs are M2 phenotype due to the character of microenvironmental cytokines, chemokines and growth factors polarizing required monocytes and residential macrophages to phenotypes beneficial for tumor cells persistence. TAMs greatly participate in all steps of tumor progression, mainly through the production of various factors involved in the extracellular matrix remodeling, promoting neovascularization, promoting tumor cells migration, intravasation and their survival in the circulation, as well as establishing the pre-metastatic niche. TAMs are also crucial player in tumor response to chemotherapy by developing tumor protective phenotype, thus limiting the effectiveness of various type of chemotherapeutic agents e.g. platinum compounds, paclitaxel, gemcitabine or doxorubicine. TAMs-mediated resistance to above mentioned agents includes, among others, the following mechanisms: i) production of cathepsin B and IL-10 responsible for inhibition of tumor cells apoptosis; ii) production of IL-6, that activates STAT3 in tumor cells promoting their survival; iii) production of various growth factors (VEGF, EGF) activating pro-survival ERK1/2 and AKT/PI3K proteins in tumor cells; iv) release of micro RNA, targeting tumor cells signaling pathways, suppressing DNA damage and apoptosis, while promoting tumor cells proliferation; v) upregulation of cytidine deaminase in tumor cells responsible for deactivation of gemcitabine. In summary, TAMs are important component of TME, contributing to tumor progression, metastasis and reducing an efficacy of conventional therapies.

The effect of cancer cells on the formation of NETs

Marzena Garley[1], Dorota Dziemiańczyk-Pakieła[2], Wojciech Miltyk[3], Kamil Grubczak[4], Arkadiusz Surażyński[5], Wioletta Ratajczak-Wrona[1], Małgorzata Grudzińska[6], Kinga H. Nowacka[1], Marcin Moniuszko[4], Jerzy A. Pałka[5], Jan Borys[7], Ewa Jabłońska[1]

1 – Department of Immunology, Medical University of Bialystok
2 – Otolaryngology and Maxillofacial Surgery Ward of the Provincial Integrated Hospital Jędrzej Śniadecki in Bialystok
3 – Department of Pharmaceutical and Biopharmaceutical Analysis, Medical University of Bialystok
4 – Department of Regenerative Medicine, Medical University of Bialystok
5 – Department of Medicinal Chemistry, Medical University of Bialystok
6 – Department of Pathomorphology, Medical University of Bialystok
7 – Department of Maxillofacial and Plastic Surgery Medical University of Bialystok

Background: Recent studies show that the interaction and modulation of the blood microenvironment by circulating cancer cells is essential for disease progression. Our previous studies demonstrated an increase in the percentage of NETs-forming neutrophils in patients with oral squamous cell carcinoma (OSCC). Inducers of NETs formation in the tumor microenvironment may be mediators released by other cells of the immune system, tissue stromal cells and tumor cells, or direct contact of neutrophils with cancer cells.

Aim: To explain the causes of increased NETosis, the experiment aimed to evaluate the process of NETs formation in patients with OSCC in response to direct or indirect contact with SCC cells in comparison to results obtained in the neutrophils of healthy subjects. To explain the mechanisms underlying the relationship between neutrophils and cancer cells, an attempt was made to analyze the mechanisms of NETs generation through the PI3K/Akt pathway.

Material and methods: As the experimental model we used the CAL 27 cell line and peripheral blood neutrophils from cancer patients and healthy people. The analysis of parameters associated with the formation of NETs after neutrophil coculture with SCC cells and after stimulation with supernatant – SCC cell culture products were analyzed by flow cytometry, fluorescence microscopy, and ELISA assays. The expression of proteins involved in the PI3K/Akt pathway in neutrophils was evaluated by Western blotting.

Result: The intensity of NETs formation in response to direct contact with SCC cells not only confirms the participation of neutrophil traps in the cancer process but also indicates the direct interactions between the cells. However, the formation of larger amounts of NETs after stimulation with the supernatant obtained from SCC cell culture may indicate the dominant role of mediators released from cancer cells in the tumor microenvironment. The enhanced process of NETosis was accompanied by changes in the proteins of the PI3K/Akt pathway, with an increase in p-Akt expression and a decrease in p-PI 3k expression in the neutrophils sorted after coculture.

Conclusions: The obtained results prove the existence of direct interactions between cancer cells and neutrophils resulting in NETosis. The discovery of the mechanism of the Akt kinase regulation, independent of PI3K, which leads to the formation of NETs could be the target of new anti-cancer immunotherapy.

21. Polish Immunology: from past to the future

The role of polish scientists in the discovery of hepatitis B virus

Kazimierz Madaliński

The National Institute of Public Health – National Institue of Hygiene; Dept. Virology; Prof. Emeritus

Several factors influenced the success of a relatively poorly equipped team of Polish scientists in discovery of the hepatitis B virus in 1970's. First, the new place of work – The National Institute of Hygiene, directed by energetic Professor Włodzimierz Kuryłowicz; second – friendly, non-competitive atmosphere in the team; and third – yearly education of each member of the team in the advanced American center – Cornell University Medical College.

The next sparkle to induce the initiative was the proposition to solve the nature of the immunochemical reaction, i,e, antigen – antibody, observed by the group of Prof. Halina Seyfriedowa from the neighboring Institute of Hematology. Immunochemical analysis In our laboratory allowed to identify the 'Australia antigen' and corresponding antibodies in the hemophilia sera. Australia antigen was of lipoprotein nature and of high molecular mass. The next question was: is the antigen-antibody system of intrinsic or extrinsic character? The next hard data were obtained by immunofluorescence and electron microscopy. After the proved finding of Australia antigen in the sera of six patients with lymphoproliferative disorders; it was next detected In the liver taken at the autopsy of the same patients. The specific immunofluorescence was localized In the cytoplasm (20 nm particles) and nuclei (27 nm) of hepatocytes. Furthermore, the chains of virus-like particles, were detected in the nuclei of hepatocytes by electron microscopy. The particles fund In the liver were evidently of extrinsic character. After the final proof of the causal relationship between the Australia antigen and acute/chronic hepatitis B, the former was correctly re-named as the hepatitis B surface antigen. The genetic copies of this antigen became the vaccine source against hepatitis B. The following achievement of the Polish group was the discovery of the role of hepatitis B virus in two extrahepatic diseases.

In summary, Polish main achievement in the discovery of hepatitis B virus was the performing broad immunohistochemical studies of the localization of viral particles in tissues. In particular, the discovery of 27-nm particles in the nuclei of hepatocvtes. strongly supported the correctness of the structure proposed as the full hepatitis B virus – Dane particle.

Neutrophils yesterday and today: soldiers and saboteurs of the immune system

Janusz Marcinkiewicz

Department of Immunology, Jagiellonian University Medical College, Krakow, Poland

Since the discovery of phagocytosis at the end of XIX century until the discovery of toll-like receptors on the cells of innate immunity (1997), neutrophils were treated as the primary executive cells of inflammation. The contemporary common opinion was that neutrophils were designed just to kill invaders without any contribution in the induction stage of the more sophisticated adaptive immunity. The aim of the presentation is to show the differences in our understanding of the role of neutrophils in the immune system before and after 1997 year. The following aspects of the research of neutrophils will be discussed:

- Milestones of neutrophil discoveries
- The discovery of TLRs on neutrophils – the breakthrough in the research of innate immunity
- A role of the MPO-halide system in innate and adaptive immunity
- A role of the neutrophil taurine in regulation of inflammation
- Neutrophil „cross–talk" with other immune cells
- Neutrophils started out the full partners of lymphocytes in the adaptive immunity
- Neutrophils as the accessory antigen-presenting cells (APC- accessory cells)
- The contribution of Kraków scientists to this idea

The role of Poznan scientists in the organization of Polish immunology

Jan Żeromski

Chair of Pathomorphology and Clinical Immunology, Karol Marcinkowski University of Medical Sciences, Poznan, Poland

The city of Poznan, population 700,000, Is a medieval town with rich historical background. It is now an important centre of Polish science with many academic institutions and several thousand students. The Faculty of medicine founded in parallel with the University 100 years ago, is known for its merits in various fields of medical sciences. Interest in immunology in the 1960s was reflected by a good scientific atmosphere in several medical departments as well as young staff members full of novel ideas. Informal discussion groups were formed in some units, devoted to talk in English about the structure and function of the immune system. At about the same time, Poznan clinicians and pathologists became involved in the study of immunoglobulins and autoantibodies in autoimmune diseases. Poznan immunologists actively participated in the foundation of the Polish Society for immunology. The Board of the Society soon moved to Poznan. All together, as many as four Poznan immunologists served as presidents of the Society. The organ of the Society Immunologia Polska was created and published as a quarterly for 20 years. Regular all- Poland meetings "Progress of Immunopathology in Clinical Diagnostics" were held every three years in Poznan with broad participation of doctors, students and diagnosticians from the whole country. Besides, at least 3 international conferences devoted to various aspects of immunology were organized in Poznan. The international prestige of Poznań immunology resulted in entrusting the Polish Society the organization of 14th European Immunology Meeting (EFIS2000) in Poznan with more than 1000 participants. It was an enormous effort and cost, fortunately generously supported by Poznan municipal authorities and commercial companies. There are several important research achievements of Poznan immunologists, but this is not the topic of the current presentation.

22. Progress in diagnostics and treatment of primary immunodeficiencies

Newborn screening for early diagnosis and treatment of severe combined immunodeficiency (SCID)

Małgorzata Pac

Department of Immunology, The Children's Memorial Health Institute, Warsaw

Newborn screening (NBS) tests enable identification of infants with life-threating disorders, requiring early intervention to save their life. The DNA-based assays detecting T-cell receptor excision circles (TREC) for T cells deficiencies, especially SCID, followed by kappa-deleting recombination circles (KREC) indicating B cells deficiencies have been successfully implemented in US and other countries since 2008.

Material and Methods: The Polish-German transborder cooperation in the field of newborn screening for SCID and T and/or B cell lymphopenia started in 2017, covering regions of West Pomerania and Mecklnmburg – Western Pomerania and part of Brandenburg. The total population in 2016 in that parts was 1 708 174, 724 161 and 300 243 respectively.

The aim of this study is to present the results of this cooperation. Between October 22, 2018 and March, 1, 2021 the TREC, KREC and ACTB tests were performed in 96 782 newborns using a commercial kit – SPOT-it™ TK (ImmunoIVD, Sweden).

Results: Out of 96 782 newborns 34 947 were from West Pomerania while 23 280 and 38 555 from Mecklnmburg – Western Pomerania and part of Brandenburg respectively. Within that groups following Inborn Errors of Immunity (IEI) were diagnosed: two SCID, two AR agammaglobulinemias , one Nijmegen breakage syndrome, one B-cell lymphopenia due to mother's immunosuppression, one T-cell lymphopenia due to preamaturity. Additionally six false positive B and/or T cell lymphopenias were found.

Conclusions: Newborn screening programs, including TREC and KREC together with detailed immunological assessment, are of great value to avoid severe complications in infants with IEI.

Genotype-phenotype correlations in primary immunodeficiencies

Beata Wolska-Kuśnierz

Immunology Department, Children's Memorial Health Institute

Immunology and genetics are non-separable fields of science that have come closer to each other in the last decade thanks to the introduction and better availability of next generation sequencing (NGS) methods. The number of new genes responsible for immunity disorders discovered every year has increased significantly, and we are also learning about new clinical phenotypes of known genes. According to the latest 2019 IUIS (International Union of Immunological Societies) classification, inborn errors of immunity (IEI) includes 430 separate syndromes, and over the last 2 years, 64 new genetic defects associated with them have been discovered. In practice, we are faced with the choice of the optimal method of genetic diagnosis, having a choice between the classical Sanger sequencing of single genes or the simultaneous assessment of thousands of genes based on NGS. The presentation briefly presents the problems we face in the interpretation of genetic tests. Examples of the phenomena of mosaicism, incomplete clinical penetration, and the influence of epigenetic factors on the clinical penetration of pathogenic variants in genes are discussed.

Advances in Flow Cytometry in Nijmegen Breakage Syndrome

Piątosa B[1], Heropolitańska-Pliszka E[2], Wolska-Kuśnierz B[2], Tkaczyk K1, Grycuk U[1], Gregorek H[3].

1 – Histocompatibility Laboratory, Childrens' Memorial Health Institute, Warsaw, Poland
2 – Department of Clinical Immunology, Childrens' Memorial Health Institute, Warsaw, Poland
3 – Department of Microbiology and Clinical Immunology, Childrens' Memorial Health Institute, Warsaw, Poland

Patients with Nijmegen Breakage Syndrome (NBS) demonstrate features of cellular and humoral immune deficiency. The product of mutated *NBN* gene affects the process of V(D)J recombination necessary for normal early T and B lymphocyte maturation, class switch recombination (CSR) in mature B cells, and repair of lesions caused by mutagenic agents and ionizing radiation. General lymphopenia observed in the patients affects both the T and B cells.

B cell maturation process occurs stepwise. The mutated nibrin affects the precursor B cell maturation in bone marrow. As result, lower numbers of transitional cells and naïve B lymphocytes ready for peripheral differentiation are generated than in healthy controls. After contact with antigens, the naïve B population seems to proliferate vigorously, as proportions of natural effectors are much higher in NBS patients than in healthy controls. During the germinal center reactions similar proportions of centroblasts and centrocytes are generated, but the class switched memory compartment is composed mainly of IgM-only memory B cells. Significantly lower proportions of IgG and IgA memory than in healthy controls, reflect aberrant V(D)J IgH rearrangement. CD27-IgG+ and CD27-IgA+ memory B cells, as well as plasma cells are produced in similar proportions as in healthy controls. Among the previously reported increased population of IgD-CD27- cells, we identified similar proportions of IgG+ and IgA+ cells as in healthy controls, and an unusual subset of IgM+CD95+CXCR3+ cells. Such phenotype has been suggested to describe a product of aberrant extrafollicular B cell differentiation.

Despite low thymic production, the maturing T lymphocytes proliferate at an increased rate in comparison to healthy controls and the process is initiated at the stem-cell like stage. As result, almost normal proportions and absolute counts of central and effector memory, and increased numbers of terminally differentiated T cells demonstrating features of pre-term senescence and exhaustion are produced. Aberrant rearrangement of TCR genes is manifested by variable defect in TCRVβ chain distribution and reduced proportions of MAIT lymphocytes defined by expression of invariable TCRVα7.2 chain.

In conclusion, we have demonstrated the effects of aberrant heavy immunoglobulin and TCR chains rearrangement. The increased proliferative potential of both T and B lymphocytes probably contributes to an increased susceptibility to malignancy in NBS patients.

Spectrum of cutaneous manifestation in a large cohort of polish patients with Nijmegen Breakage Syndrome (NBS)

Edyta Heropolitanska-Pliszka[1], Beata Wolska-Kusnierz[1], Barbara Pietrucha[1], Barbara Piatosa[2], Hanna Gregorek[3], Krystyna Chrzanowska[4], Bożena Mikołuc[5]

1 – Department of Immunology, Children's Memorial Health Institute (CMHI), Warsaw, Poland
2 – Histocompatibility Laboratory, CMHI, Warsaw, Poland
3 – Department of Microbiology and Clinical Immunology, CMHI, Warsaw, Poland
4 – Department of Genetics, CMHI, Warsaw, Poland
5 – Department of Pediatrics, Rheumatology, Immunology and Metabolic Bone Diseases, Medical University, Bialystok, Poland

Background&aim: NBS is a rare autosomal recessive DNA-repair disorder, characterized by microcephaly, facial dysmorphy, skin pigmentation defects, combined immunodeficiency, chromosomal instability, and high predisposition to malignancy. Most patients are of Slavic origin and carry the same homozygous deletion (c.657_661del5) of NBN gene. There are only a few case reports on cutaneous manifestations of NBS in literature, so we sought to delineate dermatological features of big series of Polish NBS patients.

Methods: The study was performed as a part of ERA-NET-E-Rare-3/I/EuroCID/04/2016 grant in CMHI, Warsaw. All patents had detailed cutaneous examination, other data were extracted from patients' charts.

Results: 52 patients (22M, 30F) were interviewed and examined. Median age at assessment was 11,8 years (range 6 months-39 years). Pigmentation anomalies included café-au-lait spots (91%), hypopigmented macules (52%), melanocytic nevi (48%). 39% of patients presented with persistent form of vasculitides (livedo reticularis) and Raynaud's-like phenomenon. Granulomatous skin lesions were clinically diagnosed in 23% of cases, in 15,5% being histologically confirmed. In 2 cases of atopic/eczematous-like erythroderma, skin biopsy revealed granulomatous changes. Autoimmune complications (vitiligo, alopecia) showed 5 and 2 patients, respectively. Other frequent manifestations included numerous progeric skin and hair changes. Surprisingly, any infectious skin complications were observed.

Conclusions: Rarity of disease suggests this is the largest clinical study of cutaneous manifestation involving 52 living NBS patients. Most frequent skin lesion, besides skin pigmentation defects, are form of vasculitides/Raynaud's phenomenon. Incurable, progressive skin granulomas are most challenging in diagnosis and treatment.

Atypical manifestation of APDS 1

Bohynikova N[1], Wolska- Kusnierz B[1], Dabrowska- Leonik N[1], Gladysz D[1],
Bernat- Sitarz K[1], Skomska- Pawliszak M[1], Skoczynska D[1], Piatosa B[2], Kacprzak M[3], Pac M[1]

1 – Department of Immunology, The Children's Memorial Health Institute, Warsaw, Poland
2 – Histocompatibility Laboratory, The Children's Memorial Health Institute, Warsaw, Poland
3 – Medgen Institute of Biochemistry and Biophysics, Warsaw, Poland

Activated PI3K Delta Syndrome 1 (APDS1) is recently reported new disease entity in inborn errors of immunity. It is an autosomal dominant Combined Immunodeficiency (CID) disorder caused by gain of function (GOF) mutation in the gene PI3KCD. The gene encodes p110delta catalytic subunit of Phosphoinositide 3- kinase Delta (PI3Kdelta), which has major role in proliferation, survival and activation of leukocytes. The phenotype of disease is highly variable. The patients suffer from frequent respiratory infections, severe or persistent herpersvirus infections, autoimmune diseases and manifest lymphoproliferation with increased risk of lymphomas and progressive lung disease (bronchiectasis). APDS1 has been also recognised in patients with humoral immunodeficiency and autoimmunity, previously followed up as Common Variable Immunodeficiency (CVID) or Autoimmune Lymphoproliferative Syndrome- like (ALPS-like).

Herein we report a young male patient who by the age of 4 had undergone two severe infections, moderate lymphoproliferation and some evidence of bowel involvement. The boy was admitted to our department with suspicion of leaky Severe Combined Immunodeficiency (SCID). The laboratory examination revealed profound cellular immunodeficiency with CD4+ lymphopenia and signs of immune senescence, IgG and IgA hypogammaglobulinemia and elevated IgM. The clinical diagnosis of Combined Immunodeficiency with Hyper IgM was set. Based on further clinical, laboratory and genetic testing he was finally diagnosed with APDS1. Reconsidering his history and risk of severe infections and malignancies we found him eligible for allogenic haematopoetic stem cells transplantation (HSCT) and referred him to Bone Marrow Transplant Center.

It is important to differentiate APDS from CVID due to availability of target therapy (mTOR inhibitors) and increased risk of lymphomas.

The HSCT should be considered in those patients who have severe clinical course, especially with early beginning of life- threatening events (autoimmunity, persistent infections with malignancy risk).

Diagnosis and Treatment of Patients with ADA Deficiency: a Single-Centre Experience

Dąbrowska-Leonik N[1], Bernat-Sitarz K[1], Wolska-Kuśnierz B[1], Skomska-Pawliszak M[1], Bohynikova N[1], Bernatowska E[1], Piątosa B[2], Słomińska E[3], Fabianowska-Majewska K[4], Gładysz D[1], Pac M[1]

1 – Department of Immunology, Children's Memorial Health Institute, Warsaw
2 – Histocompatibility Laboratory, Children's Memorial Health Institute, Warsaw
3 – Biochemistry Department, Medical University, Gdansk
4 – Department of Biomedical Chemistry, Medical University, Lodz

Deficiency of adenosine deaminase (ADA) manifests as severe combined immunodeficiency (SCID), caused by accumulation of toxic substrates. If left untreated patients develop immune and non-immune symptoms with fatal clinical course. According to ESID and EBMT recommendations enzyme replacement therapy (ERT) should be implemented as soon as possible to stabilize the patient's general condition, normalize transaminases, treat pulmonary proteinosis, bone dysplasia, and protect neurological damage. The target therapy is hematopoietic stem cell transplantation (HSCT) from a matched family donor (MFD). In the absence of such a donor, gene therapy (GT) should be considered. HSCT from a matched unrelated donor (MUD) and haploidentical parental transplantation are associated with a poorer prognosis.

Herein we retrospectively evaluated the clinical course and results of biochemical, immunological and genetic tests of 4 patients diagnosed in our department with ADA deficiency in the last decade.

Laboratory tests showed in every patient lymphopenia in all subpopulations of T-, B- and NK cells. Diagnosis was made on the basis of ADA activity in red blood cells and /or genetic testing. Patients had different non-immunological symptoms including: lung proteinosis, skeletal dysplasia, liver dysfunction, atypical haemolytic-uremic syndrome, psychomotor development disorders. The first 2 patients underwent successful HSCT (MFD and MUD respectively) preceded by enzyme therapy (lasting 2 and 5-month respectively). Patient 3 with multiple organ failure died shortly after admission, before the diagnosis was confirmed. Patient 4 is currently on ERT in the process of selecting an unrelated donor. No one patient had undergone gene therapy.

It is important to diagnose ADA SCID as early as possible, before irreversible multi-organ failure appears. In Poland HSCT are conducted in accordance with international immunological societies, while ERT and GT is less accessible.

23. Rheumatology

Rheumatoid arthritis as a systemic autoimmune disease – the current concept

Ewa Bryl, MD, PhD

Department of Pathology and Experimental Rheumatology

Rheumatoid arthritis (RA) is an inflammatory rheumatic disease in which the entire immune system is involved. Usually the first clinical manifestation of RA is arthritis and therefore for many years people thought that RA is only local disease. Later it was discovered that apart from arthritis/synovitis there is some general systemic inflammation – measured by elevated erythrocyte sedimentation rate or C-reactive protein in the peripheral blood of RA patients. Finally, rheumatoid factor autoantibodies were detected in peripheral circulation confirming that RA is an autoimmune disease. Knowledge regarding immune system in RA is constantly growing since many years. Currently it is recognized, that both the innate and adaptive immunity are involved in its pathogenesis, including mainly the T cells, cytokines, and autoantibodies. Increased proportion of circulating CD4+ and CD8+ T cells without expression of CD28 and increased proportion of activated CD4+ T cells circulating in RA patients are only a few examples of systemic CD4+ changes. Multiple disturbances of blood T cell homeostasis in RA patients were reported; changes include shorter telomere length, fewer recent thymic emigrants expressing T cell receptor excision circle, lower naïve T cells' antigen receptor (TCR) diversity. Circulating peripheral blood T cells of RA patients show also different functional disturbances – increased activation-induced cell death, but also changed kinetics of proliferation of the CD4+CD28+ T cells, including longer time from start of stimulation to onset of the first G1 phase, shorter duration of mitotic cycles and fewer living precursors entering divisions. Many different autoantibodies were detected in the sera of RA patients. Majority of them are produced against peptides/proteins which are post-translationally modified, mainly by citrullination, carbamylation, oxidation and acetylation. The disbalance towards more proinflammatory cytokines is pronounced. Most of the increased cytokines were associated with general immune activation, activation of Th1 and Th17 cells, and bone-derived factors. RA patients have higher levels of cytokines related to Th1, Th2 and Treg cells than control group; interestingly, chemokines, stromal cell-related cytokines, and angiogenic-related markers are increased at early stages of RA. All of these facts confirm that RA is a systemic autoimmune disease.

Involvement of innate and adaptive arms of immune system in the pathogenesis of spondyloarthritis

Maciej Siedlar, Mariusz Korkosz

Dept. of Clinical Immunology; Jagiellonian University Medical College, Cracow, Poland
Dept. of Rheumatology and Immunology; Jagiellonian University Medical College, Cracow, Poland

Spondyloarthritis (SpA) represents a group of second most prevalent inflammatory rheumatic disorders (ca. 1%) characterized by chronic inflammation and structural damage (mainly new bone formation) involving axial and peripheral skeleton. Recent research in SpA has been focused on the phenotypic presentations and pathophysiology of SpA subgroups (i.e. non-radiographic axial spondyloarthritis, ankylosing spondylitis, psoriatic arthritis, reactive arthritis, arthritis in inflammatory bowel diseases and undifferentiated spondyloarthritis) exploring, whether SpA is a single disease with various clinical expression or rather a group of distinct clinical entities sharing common signs and symptoms. Genetic, immunopathologic, and clinical evidence indicate that despite common downstream pathways, mediated e.g. by macrophage-derived TNFα and IL-17A, inflammation in SpA is driven and maintained by different cellular and molecular mediators. Moreover, it has been proposed that SpA is an autoinflammatory disease driven rather by innate immune cells, than an autoimmune disease triggered by self-reactive T and/or B lymphocytes. The phenotypic subclassification of SpA is usually based on extraarticular signs (psoriasis and inflammatory bowel disease), pathogenesis (reactive arthritis) or outcomes (ankylosing spondylitis). Nevertheless, all phenotypes share similar axial (sacroiliitis, spondylitis, back pain) or peripheral (arthritis, enthesitis, dactylitis) manifestations, and therefore SpA might be classified as one of two subforms with different pathophysiology, with predominant involvement of axial or peripheral skeleton. There is a proposal to define SpA by its pathophysiology rather than by its phenotypic presentation, since the growing evidence from immunopathology studies and clinical drug trials suggest that axial (axSpA) and peripheral (pSpA) spondyloarthritis might be driven by different mechanisms and respond differently to treatment. However, considering SpA as a possible single entity the question remains whether shared clinical features of axSpA and pSpA could have common triggers, particularly during the early phase before chronic compensatory and therapy effects occur. They are likely to be most variable and at the same time most informative at an early stage of the disease.

Epigenetic mechanisms in autoimmune response: role of miRNA

Marzena Ciechomska[1], Bartosz Wojtas[2], Krzysztof Bonek[1], Leszek Roszkowski[1], Piotr Gluszko[1], Vladimir Benes[3], Wlodzimierz Maslinski[1]

1 – National Institute of Geriatrics Rheumatology and Rehabilitation, Warsaw, Poland
2 – Nencki Institute of Experimental Biology, Warsaw, Poland
3 – European Molecular Biology Laboratory, Heidelberg, Germany

Introduction: Unique epigenetic patterns including miRNA aberration contribute to the pathogenesis of autoimmune rheumatic diseases including rheumatoid arthritis (RA) and systemic sclerosis (SSc). Monocytes play an important role in RA and SSc, since they are the first immune cells which migrate from blood to the site of inflammation leading to tissue destruction due to enhanced proinflammatory cytokines secretion. Thus, the aim of this study was to investigate whether miRNA and transcriptomic dysregulation in monocytes contribute RA and SSc pathogenesis.

Methods: Next generation sequencing (NGS) for transcriptome analysis (RNA-seq) and for miRNome analysis (miRNA-seq) was performed simultaneously on healthy control (HC), SSc and RA monocytes. Hierarchical clustering was implemented in order to select dysregulated genes and reversely expressed miRNA, which are predicted to be the putative target genes. Following computational analysis, selected miRNAs-mRNA candidates were validated using qPCR and correlated with clinical parameters and functional assays were performed.

Results: Our results from NGS and qPCR analysis confirmed aberrant pro- and anti-inflammatory genes expression in RA and SSc monocytes. In particular, IFN-regulated genes (IRGs) based on IFIT1, IFIT3, IRF7, Siglec1, IFI44, IFI44L were significantly increased, whereas miRNA-26a which is predicted to regulate selected IRGs was significantly reduced in SSc monocytes. On the other hand, anti-inflammatory RARA was significantly reduced, whereas miRNA-146b which is predicted to regulate RARA was significantly increased in RA monocytes. Additionally, miRNA-146b expression was significantly correlated with clinical parameters including disease activity score-28. Functional assays also confirmed that overexpression of miRNA-146b or miRNA-26a in THP-1 monocytic cell line was able to functionally reduce RARA or IRGs expression, respectively. Finally, circulating miRNA-146b expression in sera and synovial fluids was significantly elevated in RA patients.

Conclusions: Overall, these studies may open new possibilities for the development of novel epigenetic miRNA-mediated molecules regulating pro-inflammatory cytokines production. Furthermore, selected miRNA candidates may be used as diagnostic biomarkers that monitor the response to therapy in rheumatic diseases.

Supported by 2018/30/E/NZ5/00104 from National Science Centre.

Association of surface expression of the CD94 and NKG2D receptors with rheumatoid arthritis

Milena Iwaszko[1], Agnieszka Chrobak[2], Jerzy Świerkot[3], Piotr Wiland[3], Katarzyna Bogunia-Kubik[1]

1 – Laboratory of Clinical Immunogenetics and Pharmacogenetics, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland
2 – Laboratory of Reproductive Immunology, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland
3 – Department of Rheumatology and Internal Medicine, Wrocław Medical University, Wrocław, Poland

Rheumatoid arthritis (RA) is a multisystem autoimmune disease characterized by inflammatory synovitis leading to joint destruction. Natural killer (NK) cells are involved in pathogenesis of the RA. The function of these cells is modulated by CD94/NKG2 receptors belonging to the C-type lectin-like family. These molecules mediate either the inhibition or the augmentation of cytotoxicity, as well as the generation of proinflammatory cytokines by NK cells. An imbalance in cytotoxic activity and cytokine production has been implicated in pathogenesis of the RA. Therefore, the CD94/NKG2 receptors may play a potential role in the RA development.

The aim of this research was to examine a potential relationship between protein expression levels of the CD94/NKG2 receptors on NK cells and the RA development. A total of 34 patients diagnosed with the RA from Department of Rheumatology and Internal Medicine of Wroclaw Medical University were enrolled to the study. The control group consisted of 24 healthy blood donors recruited from Blood Bank of Wroclaw. A number of CD3-CD56+ cells expressing the CD94, NKG2A, NKG2C and NKG2D molecules was measured using the flow cytometry.

Significant differences between the patients and healthy controls have been detected with regard to surface expression of receptors from the CD94/NKG2 family. The percentage of CD3-CD56+ cells expressing the NKG2D receptor was significantly higher in the RA patients as compared to the healthy controls ($p = 0.021$; $W = 555.0$). In addition, a significant increase in the frequency of CD3-CD56+ cells positive for the CD94 molecule was observed in the RA patients in comparison to the control group ($p = 0.035$; $W = 542.0$). There were no significant differences in the frequency of CD3-CD56+NKG2A+ as well as CD3-CD56+NKG2C+ cells between the patients and healthy individuals. The obtained results suggest a potential role of the NKG2D receptor as well as the CD94 molecule in pathogenesis of the RA.

This work was supported by the National Centre of Science (Poland) – grant no. 2016/21/B/NZ5/01901.

Can miRNA profiling be helpful in distinguishing rheumatic disorders?

Chaszczewska-Markowska Monika[1], Gebura Katarzyna[1], Swierkot Jerzy[2],
Sokolik Renata[2], Bugaj Bartosz[2], Bogunia-Kubik Katarzyna[1]

1 – Laboratory of Clinical Immunogenetics and Pharmacogenetics, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland.
2 – Department of Rheumatology and Internal Medicine, Wroclaw Medical University, Wroclaw, Poland.

Rheumatoid arthritis (RA), psoriatic arthritis (PsA) and ankylosing spondylitis (AS) are systemic, inflammatory autoimmune diseases, affecting considerable percentage of population. Pathological mechanisms behind these diseases remain unclear. MicroRNAs (miRNAs), which are known to be potent regulators of inflammatory responses, may play an important role as factors potentially affecting disease susceptibility and progression.

Our current study revolved around characterization as well as differences in miRNA profiles between RA, PsA and AS patients (N=4 for each group). Results obtained for each group of patients were compared to those for healthy controls (N=4). Peripheral blood was collected and total RNA including miRNA was extracted using Tempus miRNA system (Life Technologies). Then, after confirmation of obtained yields (4200 TapeStation, Agilent) and integrity measured by RNA Integrity Number (RIN) (2100 Bioanalyzer, Agilent), miRNA profiles of studied individuals were analysed with Agilent miRNA Microarray System.

Analysis of obtained data revealed that expression of 467 miRNAs was significantly different between healthy controls and patients with RA, on probability (p) level of 0.05. As for PsA and AS patients, expression of respectively 985 and 76 miRNAs was different when compared to the control group, at $p<0.05$. In turn, when $p<0.01$ was considered, 51, 256 and 5 miRNAs were found to be expressed differently in RA, PsA and AS patients, respectively, when compared to healthy individuals.

Interestingly, patients with RA differed from those with PsA in expression levels of 514 (at $p<0.05$) or 84 (at $p<0.01$) miRNAs. This finding is worth mentioning, as rheumatoid arthritis is considered to have very similar inflammatory and molecular background to psoriatic arthritis.

Summarizing, our current results may greatly improve our understanding of mechanism of development of RA, PsA and AS.

This work was supported by the National Science Centre (Poland) grant: 2016/21/B/NZ5/01901.

24. The expanding field of secondary immune deficiencies in adults: causes, diagnosis, and management

Immune-related adverse events of cancer therapies

Jacek Roliński

Department of Clinical Immunology, Medical University of Lublin, Chodzki 4a, 20-093 Lublin, Poland

Immune checkpoint inhibitors (ICIs) are increasingly used for treatment certain cancers. ICIs including anti-CTLA-4 (cytotoxic T lymphocyte antigen-4) and anti-PD-1/PD-L1 (programmed death-1/programmed death-ligand 1) have increased overall survival for patients with various cancers. ICIs increases antitumor immunity, however, this has resulted in increased reports of immune-related adverse events (irAEs). With the increasing use of ICIs alone or in combination with other therapies, awareness and management of immune-related adverse events (irAEs) have become more important. The precise pathophysiology of irAEs is unknown. Studies have shown different mechanisms of irAEs that T-cells, antibodies, and cytokines response may be involved. It is not known why they occur only in some patients. IrAEs usually start in the first few weeks or months after treatment but can occur even after the end of treatment. Glucocorticoids are usually the first-line immunosuppressive agent. More data are needed to address whether the occurrence of irAEs is associated with improved treatment efficacy.

25. Tumor Immunology and Tumor Immunotherapy

Evolving role of the immune system in the process of cancer development

Witold Lasek

Department of Immunology, Warsaw Medical School, Warsaw, Poland

Advances in molecular and cellular immunology at the end of the 20th century clearly showed that the immune system plays an important role in carcinogenesis and tumor development. In 2001, the concept of cancer immunoediting was proposed, strongly supported by results from animal models of carcinogenesis and clinical observations. This concept confirmed and completed the hypothesis of cancer immune surveillance, that was formulated in the 1950s.

The hypothesis of cancer immunoediting summarizes the relationship between the immune system an transformed/neoplastic cells in three steps: elimination, equilibrium, and escape. Over the years, the immune system can repeatedly recognize and kill the emerging transformed cells (elimination phase). In some cases, the carcinogenesis process continues and the effector elements of the immune system are unable to completely eradicate the transformed or tumor cells (equilibrium phase). Finally, under certain circumstances, due to frequent mutations in tumor cells and changes in their antigenicity, the process of equilibrium turns into overt tumor development (escape phase), which ultimately leads to the death of the host.

The immune system plays a different role in each phase of cancer immunoediting. In the elimination phase, specific immune mechanisms dominate, while in the step of escape, the role of the immune system is more complex, based on both innate and adaptive response. In the latter phase, tumor growth is associated with a continuous modification of the microenvironment. Changes in the tumor microenvironment and the direct influence of neoplastic cells on the effector mechanisms of the immune system lead to situations that favor and support the development of cancer. This phenomenon is in part related to the development of cells such as Treg lymphocytes, M2 macrophages, and tolerogenic dendritic cells, which create a tumor-promoting immunosuppressive milieu.

Therapeutic gene modified melanoma stem cell-like vaccine represses T cell exhaustion, maintains T cell stemness, and enhances T cell chemotaxis

Patrycja Czerwińska[1,2], Nikola Agata Włodarczyk[1], Anna Maria Jaworska[1], Marcin Ruciński[2], Katarzyna Gryska[1], Mariusz Kaczmarek[1], Andrzej Mackiewicz[1,3]

1 – Department of Cancer Immunology, Chair of Medical Biotechnology, Poznan University of Medical Sciences, Poznan, Poland
2 – Department of Histology and Embryology, Poznan University of Medical Sciences, Poznan, Poland
3 – Department of Diagnostics and Cancer Immunology, Greater Poland Cancer Centre, Poznan, Poland

We developed a therapeutic, gene-modified, allogeneic melanoma vaccine (AGI-101H), which, upon genetic modification, acquired melanoma stem cell-like phenotype. Since its initial clinical trial in 1997, the vaccine has resulted in the long-term survival of a substantial fraction of immunized patients (up to 21 years). Here, we investigated the potential molecular mechanisms underlying the long-lasting effect of AGI-101H using transcriptome profiling of patients' peripheral T lymphocytes.

Magnetically separated, untouched peripheral T cells from AGI-101H-immunized long-term survivals (at day 6 and 11 after vaccination), untreated melanoma patients and healthy controls were subjected for transcriptome profiling using HG U219 microarrays. Data were analyzed with bioinformatics tools (DAVID, GSEA) and validated with RT-qPCR and FACS analyses.

AGI-101H treatment activated the TNF-α and TGF-β signaling pathways and dampened IL2-STAT5 signaling in T cells six days after vaccine administration. It resulted in the significant up-regulation of a BCL6 transcriptional repressor and corresponding down-regulation of several Bcl6 target genes and exhaustion markers, suggesting that Bcl6 may facilitate the progenitor-fate of cancer-experienced T cells in AGI-101H-vaccinated patients. Relatively to the day of vaccine administration (day 0), we observed significant enrichment of Reactome terms associated with the regulation of gene expression at day 6. Among the top enriched Reactome terms at day 11, we identified significant enrichment of signaling by interleukins and chemokines. A closer look at the chemokine profiles characterizing peripheral T cells revealed small although statistically significant up-regulation of 6 markers (CCL13, CXCL5, CXCL16, CCR1, CCR3, CCR6) and down-regulation of 2 markers (CKLF, CCR10) at day 6 upon AGI-101H vaccination. On the contrary, at day 11, we observed robust up-regulation of 18 markers encoding either chemokines or chemokine receptors (i.e., CCL3, CCL4, CCL20, CXCL2, CXCL3, and others). We also observed various profiles of expressed interleukins at day 6 and day 11 after AGI-101H administration, with tremendous up-regulation of IL1A, IL1B, and IL8 (CXCL8) at day 11.

AGI-101H mobilizes peripheral T cells to protect against further tumor development in melanoma-experienced patients, at least partially by repressing T cell exhaustion and subsequent induction of lymphocyte migration.

PD-1/PD-L1,L2 pathway as a target for lung cancer therapy

Joanna Domagała-Kulawik

Department of Internal Medicine, Pulmonary Diseases and Allergy, Medical University of Warsaw

PD-1/PD-L1,L2 pathway belongs to strong suppressors/regulators of immune system. PD-1 molecule is preferentially expressed on activated T lymphocytes, also on B cells. The PD-1 ligands: PD-L1 is expressed on epithelial and non-epithelial cells, PD-L2 on non-epithelial cells: dendritic cells (DCs) and macrophages. PD-L1 is overexpressed on cancer cells. The ligation of PD-1 with PD-L1 causes suppression of the function of cytotoxic lymphocytes. PD-1/PD-L pathway is involved in regulation of many kinds of immune response like autoimmunity and infections, but the most important is regulation of tumor immunity. In our studies an elevated expression of PD-1 on activated and memory CD8+ cells in the bronchoalveolar lavage fluid (BALF) of patients with lung cancer was observed. Recently, we showed the difficulties in quantitative analysis of PD-L1, PD-L2 expression on BALF macrophages. In the other studies we detected PD-L1 expression on cancer stem cells (CSCs) in biopsy from metastatic lymph nodes in lung cancer (EBUS/TBNA). The more, we found that PD-L1+ CSCs were capable of modify immune anticancer reaction influencing results of therapy. The blockade of PD-1 was shown to have strong therapeutic potential and belongs to immune check point inhibitors (ICIs). The use of ICIs opened a new era in the therapy of advanced solid tumors. Lung cancer is a leading malignant disease in morbidity and mortality. More than 70% of cases are recognised in advanced stages, which need qualification to systemic therapy including immunotherapy. PD-L1 expression on cancer cells is to date only validated biomarker for anti PD-1 therapy. PD-L1 could be detected in cancer tissue and liquid biopsy. However, there are some controversies concerning PD-L1 examination including: technical dilemma, quality of the tests, omission of PD-L2 activity, heterogeneity of cancer cells, dynamics of PD-L1, L2 expression. Recently published results of lung cancer immunotherapy showed an important long term-survival (up to 70 months) benefit but only in less than 40% of patients. There are some mechanisms of resistance to ICIs: lack of immunogenicity, enhanced T cell exclusion, lack of response to IFNγ, upregulation of other immunosuppressive receptors on T cells: CTLA-4, TIM3, LAG-3, VISTA or low neoantigens production. Finally, PD-1/PD-L1 inhibitors cause specific immune-related adverse events developing on the basis of autoimmune inflammation of many organs.

Tumor microvesicles interactions with endothelial cells – understanding tumor metastasis

Aleksandra Bielawska-Pohl, Agnieszka Krawczenko, Elżbieta Wojdat, Aleksandra Klimczak

Laboratory of Biology of Stem and Neoplastic Cells, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland

Introduction: Primary tumors show a tend to metastasize to specific secondary sites. Recently, the relation between metastatic organotropism and cancer microvesicles has been explored. Primary tumor cells circulating in the blood must interact with endothelial cells to arrest and extravagate into metastatic organs. Therefore, it is important to investigate the biological activity of endothelial cells (ECs) isolated from different target metastatic organs activated with tumor-derived microvesicles.

Materials and methods: The organ-specific vessel EC lines used in this work were of human brain (HBrMEC) and lung (HLMEC) microvasculature origin and HUVEC cells as a model of reference of Ecs. Tumor microvesicles (TMVs) were isolated from human breast cancer MDA-MB-231 and human melanoma Hs294T cell lines using sequential centrifugation. After characterization and enumeration of TMVs the proliferation of Ecs cultured in the presence of TMVs were examined (MTT assay). Next, adhesion (flow cytometry) and migration (experiments with transwell inserts) of tumor cells towards Ecs activated by TMVs were estimated. Finally, the secretion profile of Ecs treated with TMVs were investigated using protein membrane array analysis.

Results: The proliferation of tissue-specific EC stimulated by TMVs was higher only at the day 4th of stimulation compared to the control cells. Generally, tumor cells adhesion remained more efficient to tissue-specific EC treated with TMVs compared to unstimulated Ecs. There was no difference in the adhesion of Hs294T cells towards HBrMEC after stimulation by TMVs. Moreover, tumor cells preferentially migrated towards Ecs treated with TMVs. The most effective migration was observed for MDA-MB-231 cells towards Ecs of brain origin after TMVs stimulations. Ecs after stimulation by TMVs secreted higher level of ICAM-1, RANTES, SDF-1, VEGF-D and Angiogenin-2 proteins compared to control. Additionally, HUVEC cells treated with TMVs reduce VE-cadherin expression level leading to transformation into tumor endothelial cells.

Conclusions: It was confirmed that MDA-MB-231 MVs preferentially activate Ecs of brain origin, according to the patterns of metastatic spread of breast cancer tumors. Moreover, secretion profile of EC of brain and lung origin treated with TMVs showed that TMVs are responsible for tumor angiogenesis (augmentation of VEGF-D, Angiogenin-2). TMVs were also enable to induce endothelial-mesenchymal transition in HUVEC cells.

Alarmin HMGB1 affects the proinflammatory response of mast cells

Justyna Agier[1], Ewa Brzezińska-Błaszczyk[1], Elżbieta Kozłowska[1],
Paulina Żelechowska[1], Sylwia Różalska[2]

1 – Department of Experimental Immunology, Medical University of Lodz, Lodz, Poland
2 – Department of Industrial Microbiology and Biotechnology, University of Lodz, Lodz, Poland

Background. Alarmins are small endogenous immune-activating molecules released in response to cell injury or death. Research of alarmins has established that these homeostatic intracellular proteins can have additional, non-homeostatic functions that signal danger and promote inflammation; therefore, they are also called danger-associated molecular patterns (DAMPs). One representative of alarmins is high-mobility group box 1 (HMGB1), passively released from the nucleus into the extracellular space immediately after injury. Mast cells (MCs) are the primary effectors of inflammation; they can be called cellular sensors to initiate an appropriate physiological response either aimed to promote inflammation for repair or, on the other hand, limit the inflammatory process to avert further damage. However, data relating to the impact of HMGB1 on MCs activity is not entirely known.

Aim. The study aimed to determine whether HMGB1 affects the inflammatory response of MCs, including cytokine/chemokine/reactive oxygen species (ROS) production and cell migration.

Materials and methods. All experiments were carried out *in vitro* on freshly isolated peritoneal MCs obtained from female albino Wistar rats. qRT-PCR and ELISA were used to determine constitutive and HMGB1-induced cytokine/chemokine mRNA and protein levels in MCs, respectively. ROS generation by HMGB1-induced MCs was measured spectrometrically. In addition, MC migratory response to HMGB1 was examined *in vitro* using Boyden microchamber assay.

Results. Among the cytokines/chemokines measured in HMGB1-stimulated MCs, the highest mRNA expression levels were observed for IL-1β, CCL3, TNF, and TGF-β. Alarmin also induced a considerable release of TNF and IL-1β proteins from MCs. Results also indicate that HMGB1 strongly influenced MC ROS generation and promoted their migration.

Conclusion. Our observations show that used alarmin may stimulate MCs to release pro-inflammatory and immunoregulatory mediators and induce a migratory response. Our results highlight that HMGB1 might strongly influence MCs' activity, mainly by strengthening their role in the inflammatory mechanisms and controlling the activity of cells participating in inflammation.

This work was supported by the National Science Centre in Poland (no 2018/02/X/NZ6/03190).

Comparison of Tumor-derived Extracellular Vesicles (TEVs) isolation methods from culture supernatant for better yield, purity and unchanged biological properties

Anna Alwani, Aneta Andreasik, Kazimierz Węglarczyk, Monika Baj-Krzyworzeka

Department of Clinical Immunology, Jagiellonian University, Medical College, Kraków

Extracellular vesicles (EVs) comprise a heterogeneous group of lipid-bilayer enclosed vesicles released by all cells, including neoplastic cells (TEVs, tumor-derived EVs). According to the guidelines of MISEV2018, the gold standard for EVs isolation is ultracentrifugation (UC). Recently, many research groups introduced size exclusion chromatography (SEC) as an alternative method for EVs isolation. This study aimed to compare these two methods for isolation of EVs derived from colon cancer cell lines in terms of i). efficiency, ii). quality of EVs according to ISEV requirements and iii). phenotype.

TEVs were isolated from colon cancer cell lines obtained from ATCC (HCT116 and SW620). Cell were cultured according to the ATCC recommendation in media supplemented with exosome-depleted FBS. Cell culture supernatants were collected, filtered and used for TEVs isolation by UC or SEC method. For UC isolation, each supernatant was subjected to sequential centrifugation steps at 500xg, 35000xg and 100000xg. For SEC method, TEVs were isolated by commercially available columns (qEV 70nm, IZON).

Size distribution and concentration of TEVs were determined by Nanoparticle Tracking Analysis (NTA, Malvern Instruments Ltd). EVs specific markers (CD9, Alix) were detected by Western Blotting. Phenotype analysis was done by flow cytometry (FACS Canto II) with fluorescently labeled monoclonal antibodies anti EpCAM, CD44, CD44v6, Her-2, cMET and CD47.

The size of TEVs isolated by UC and SEC was comparable, as shown by NTA analysis (mean, D90), however, those isolated by SEC were more homogenous. The efficiency of UC isolation was higher than that of SEC, but not statistically significant. All TEVs were positive for CD9 and Alix. All TEVs express CD44, CD44v6, cMET and CXCR4 molecules, however, differences in the percentage of positive TEVs were observed. TEVs did not express EpCAM, Her-2, and CD47 markers found in abundance on cells of origin.

In summary, presented results confirm that TEVs isolated by UC and SEC methods meet MISEV2018 requirements for small EVs. However, based on the obtained results, it is strongly advised that the selection of the TEVs isolation method should consider the downstream experiments for which the TEVs are being isolated.

Coriolus versicolor fungus-derived protein-bound polysaccharides as a potential inhibitor of invasion and migration of breast cancer cells

Cybulska K.[1], Waga D.[1], Pawlikowska M.[1], Wrotek S.[1]

1 – Department of Immunology, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University, Lwowska 1 Street, 87-100 Torun, Poland

Protein-bound polysaccharides (PBP) isolated from Chinese fungus *Coriolus versicolor* are bioactive compounds that can interact with elements of the immune system and have immunomodulatory properties. Interestingly, PBP started to be used in oncology as complementary therapy for cancer patients already receiving chemotherapy or radiotherapy in China and Japan. Although, beneficial effects of PBP have been observed many times, the molecular events underlying its anticancer action are poorly understood. Therefore, the aim of the study was to test the effect of PBP on the viability, migration and invasion abilities of MCF-7 breast cancer cells.

The MTT assay and flow cytometry analysis were used to estimate the influence of PBP on the viability and cell cycle distribution in breast cancer cells, respectively. Briefly, the MCF-7 cells were stimulated with PBP extract (25, 50, 100, 150 and 200 ug/ml) and after 24-, 48- and 72 hours the viability was analyzed using MTT method. The potential influence of PBP on cell cycle of MCF-7 cells was analyzed by flow cytometry after 48 hours of cells stimulation with PBP extract at tested concentrations (25, 50, 100, 150 and 200 ug/ml). The wound healing assay was used to estimate the effect of the PBP extract on the migration of MCF-7 cells. For this test the cells were stimulated with PBP extract (25, 50, 100, 150 and 200 ug/ml) by 24 hours. In order to analyze the influence of PBP on the ability of breast tumor cells to invade the endothelium, the tumor cell transendothelial cell migration assay, based on Boyden chamber system, was performed.

The results revealed that PBP extract significantly decreased the MCF-7 cells viability, especially at concentration 150 and 200 ug/ml. Flow cytometry analysis of propidium iodide-stained cells showed that PBP extract, at concentrations of 100-, 150- and 200 µg/ml, induces S/G2-M cell cycle arrest, when compared to control cells.The results of wound healing assay proved that the PBP extract reduces the migration of breast cancer cells. The analysis revealed also that PBP extract can reduce transendothelial migration of MCF-7 cells.

In conclusion, these results clearly show that PBP extract has an inhibitory effect on the viability, migration and invasion of breast cancer cells and therefore might be consider as a drug which potentially prevents metastasis.

Administration of IL-12 and IL-18 transduced DCs can trigger the systemic anti-tumor response

Jagoda Mierzejewska[1], Katarzyna Węgierek-Ciura[1], Agnieszka Szczygieł[1], Anna Wróblewska[1], Bożena Szermer-Olerarnik[1], Joanna Rossowska[1], Elżbieta Pajtasz-Piasecka[1]

1 – Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland

Dendritic cells (DCs) genetically modified to produce cytokines such as IL-12 and IL-18, may promote the activation of anti-tumor response. IL-12 stimulates the proliferation and activates the functions of NK cells, cytotoxic T lymphocytes, and Th1 cells, and thus the secretion of IFN-γ by these cells. IL-18 even more strongly than IL-12 affects the IFN-γ production by T cells and NK cells. The synergistic action of these two cytokines leads to the induction of Th1-type immune response. The main purpose of our study was to determine the effect of administration of IL-12 and IL-18 transduced DCs on the creation of systemic antitumor response.

On the 15th, 22nd, and 29th days, DC genetically modified for production of IL-12 and IL-18 alone or as well as co-production were administered peritumorally (p.t.) to C57BL6 female mice with established MC38 tumors. On the 29th and 36th day, spleens were collected from mice to perform wide-ranging flow cytometric analyses of myeloid and lymphoid cells among splenocytes.

We demonstrated a reduction in the percentage of Treg lymphocytes and M-MDSC cells and an increase in the percentage of CD4^{+} T lymphocytes, NK, and NKT cells among splenocytes obtained from mice treated with IL-12 and/or IL-18 transduced DCs. Moreover, among the restimulated splenocytes harvested from mice receiving transduced and stimulated dendritic cells, an increase in the percentage of CD4^{+}, CD8^{+} cells, and NK splenocytes expressing upregulation of CD107a – a marker of their cytotoxic activity was noted. In supernatants from 5-day mixed culture of splenocytes and MC38 cells increased concentration of IFN-γ in groups of mice treated with DC/IL-12/TAg and DC/IL-18+IL-12/TAg and increased concentration of IL-4 and IL-10 in the group of mice receiving DC/IL-18/TAg was observed.

In summary, the use of IL-12 and/or IL-18 transduced DCs in MC38 cancer therapy significantly affects the formation of a systemic antitumor response.

This study was funded by National Science Centre, Poland (project no 2015/17/N/NZ4/02834).

The role of APRIL in the proliferation and survival of squamous cell carcinoma cells

Kinga Henryka Nowacka[1], Marzena Garley[1], Agnieszka Iwaniuk[1], Ewa Jabłońska[1]

1 – Department of Immunology, Medical University of Bialystok, J. Waszyngtona 15A, 15-269 Bialystok, Poland.

Background: The APRIL molecule, produced by several immune cells, their precursors, and cancer cells, is one of the important factors that influences the process of survival and proliferation of cancer cells. The PI3K/Akt pathway modulate proteins regulating the cell cycle and tumor invasiveness. Survivin, belonging to the IAP (Inhibitors of apoptosis), is a protein, the lack of which disrupts cell division, leading to cell death. Many types of malignant tumors are capable of express Survivin. Squamous cell carcinoma (SCC) undergo apoptosis through the involvement of both the receptor pathway and the mitochondrial pathway. The intensity of these processes depends both on the elements of the tumor cells microenvironment, expression of specific surface receptors on tumor cells, activation of various signaling pathways, and the relationship between pro- and anti-apoptotic proteins.

In the present study, we examined the effect of the rhAPRIL molecule on the proliferation and viability of cells of the squamous cell carcinoma. We traced the signal transduction that is induced in a tumor cell under the influence of rhAPRIL. We selected and studied a signaling pathway that could induce the process of cell survival in response to the action of APRIL.

Methods: SCC cells (FaDu) cultured in EMEM medium with 10% FBS Good and 1% Penicillin–Streptomycin antibiotic in a 5% CO_2 environment in an incubator at 37°C. Next, we incubated cells with or without rhAPRIL protein (100 ng/ml) by 24 or 48 hours. We measured apoptosis by flow cytometry, cell proliferation by MTT assay, and by flow cytometry using CFSE staining. The expression of signaling pathway and apoptotic proteins were examined by Western blotting.

Results: In SCC cells incubated with rhAPRIL apoptosis was decreased, while the survival and proliferation were increased, in comparison with non-exposed cancer cells. Western blot results show a difference in the induced signalling pathways in SCC cells depending on the duration of rhAPRIL exposure. In both 24- and 48-hours incubations, the expression of Akt and Survivin increased, while expression of Bak protein decreased. After 24-hour incubation, expression of caspase 3 and Bax protein were decreased. In contrast, 48-hour incubation led to decreased expression of caspase 9 and increased expression of the PI3K, Bcl-2 and Bcl-xL.

Conclusion: RhAPRIL regulates the apoptosis of SCC cells via modulation of PI3K/Akt/Survivin pathway in the time-dependent manner.

Extracellular vesicles from colorectal cancer patients act as an inducer of myeloid-derived suppressor cells

Izabela Siemińska[1], Kazimierz Węglarczyk[1], Mateusz Rubinkiewicz[2], Antoni Szczepanik[3], Maciej Siedlar[1], Jarek Baran[1]

1 – Department of Clinical Immunology, Chair of Clinical Immunology and Transplantation, Institute of Paediatrics, Jagiellonian University Medical College, Krakow, Poland
2 – Second Department of General Surgery, Jagiellonian University Medical College, Krakow, Poland
3 – First Department of General, Oncological & Gastroenterological Surgery Jagiellonian University Medical College, Krakow, Poland

Background: According to the World Cancer Research Fund colorectal cancer (CRC) is the third most common cancer in the world and in 2018 more than 1.8 million new cases of CRC were detected. Research on the mechanisms by which the developing tumor may escape the immune system led to pointing out the role of extracellular vesicles (EVs). EVs are produced by all cell types, including cancer cells, and are used for communication with other cells in the body. In case of cancer, one of the goals of such communication is an enhancement of immunosuppression supporting cancer development, which include, among others, the induction of myeloid-derived suppressor cells (MDSCs). The relevant factors responsible for the induction of MDSCs may be proteins from the Bone Morphogenic Protein family (BMP) transported by tumor-derived EVs.

Materials and Methods: EVs were isolated by sequential centrifugation from plasma samples collected from 32 CRC patients and 19 healthy donors (HD), followed by their quantitative and qualitative characterization. Next, EVs were added to monocytes isolated from HD blood by counter-current centrifugal elutriation and cultured for 24h. Thereafter cells were analyzed for mRNA expression of the BMP family proteins, PD-L1 and iNOS. Simultaneously, a measurement of cell oxygen metabolism using Mito Stress Test (Seahorse) was performed. After 24h, the cells with phenotype of Mo-MDSCs (LIN-HLA-DR-CD11b+CD15-CD14+PD-L1+), developed from monocytes cultured with EVs, were isolated by FACS-sorting and added to the cultures of stimulated autologous T cells, for assessing their immunosuppressive potential.

Results: Peripheral blood of CRC patients contains significantly more EVs than blood of HD. These EVs are positive for the expression of CD9, GM130, CD63, Hsp70, Alix, EpCAM, and Her2/neu markers, albeit the expression of Her2/neu and EpCAM is higher in the case of EVs from CRC patients. After the culture with EVs from CRC patients an increased expression of BMP1b, BMP2, iNOS, PDL-1 is detected in HD monocytes at the mRNA level. Moreover, the observed changes in oxygen metabolism indicate the induction of cells with anti-inflammatory potential. Also, the level of PD-L1+ Mo-MDSC increase after culture with CRC EVs but not from HDs. This population has shown to be suppressive in vitro for autologous T cells.

Conclusion: EVs could be responsible for induction from healthy donor monocytes a population of PD-L1 positive Mo-MDSC with suppressive activity, similarly to MDSCs isolated from CRC patients' blood. The mechanism of MDSCs induction may involve the proteins from the BMP family, including those transported by EVs.

Perspectives for the application of DCs genetically modified for overproduction of IL-12, IL-15, and IL-18 in anti-cancer therapy – *in vitro* studies

Katarzyna Węgierek-Ciura[1], Jagoda Mierzejewska[1], Agnieszka Szczygieł[1], Anna Wróblewska[1], Bożena Szermer-Olearnik[1], Joanna Rossowska[1], Elżbieta Pajtasz-Piasecka[1]

1 – Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland

Cytokines are small, secreted proteins released by cells that have a specific effect on the interactions between cells. IL-12, IL-15, and IL-18 are pleiotropic cytokines involved in the regulation of innate and acquired immune responses. IL-12 is a strong stimulator of IFN-γ production and a potent inducer of CD4 T helper (Th) 1 lymphocyte, while IL-15 and IL-18 can stimulate NK cell proliferation. Moreover, these cytokines can act synergistically increasing the immune reaction. The aim of the study was to evaluate the anti-tumor activity of IL-12, IL-15, or IL-18 produced by genetically modified dendritic cells additionally stimulated with antigens (TAg, tumor antigens).

Bone marrow-derived dendritic cells (BM-DC) were transduced with lentiviral vectors carrying IL-12, IL-15, or IL-18 genes. Anti-tumor activity of transduced BM-DC were evaluated based on cytokine production (ELISA assay), expression of DC surface markers (flow cytometry) and changes in the percentage of splenocytes as well as their cytotoxic activity after 5-days co-culture with DCs.

The production of IL-12, IL-15, or IL-18 by BM-DC transduced with lentiviral-mediated cytokine gene was confirmed and followed by the highest expression of MHC II molecule on DC/IL-12/TAg + DC/IL-15/TAg. Mixed co-cultures of splenocytes and BM-DC producing of IL-12 resulted in an increase in the percentage of $CD8^+$ and NK splenocytes expressing upregulation of CD107a. Moreover, splenocytes preincubated with DC/IL-12/TAg + DC/IL-15/TAg or DC/IL-12/TAg + DC/IL-18/TAg released higher amounts of IFN-γ as well as IL-10 than other groups.

The obtained results reveal that the enhanced production of IL-12 or IL-15 or IL-18 by DCs increases activation of splenocytes and, consequently, generates a high level of their TAg-specific cytotoxicity.

This study was funded by National Science Centre, Poland (project no 2017/27/B/NZ6/02702).

Genetics of non-small cell lung cancer: impact of antigen-processing machinery polymorphisms on disease risk and clinical parameters in smokers and never-smokers

Andrzej Wiśniewski[1]*, Maciej Sobczyński[2], Konrad Pawełczyk[3], Irena Porębska[4], Monika Jasek[1], Marta Wagner[1], Wanda Niepiekło-Miniewska[1], Aneta Kowal[4], Joanna Dubis[5], Natalia Jędruchniewicz[5], Piotr Kuśnierczyk[1]*

1 – Laboratory of Immunogenetics and Tissue Immunology, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland
2 – Department of Bioinformatics and Genomics, Faculty of Biotechnology, University of Wrocław, Wrocław, Poland
3 – Department and Clinic of Thoracic Surgery, Wrocław Medical University, Wrocław, Poland
4 – Department of Pulmonology and Lung Oncology, Wrocław Medical University, Wrocław, Poland
5 – Research and Development Centre, Regional Specialist Hospital in Wrocław, Wrocław, Poland

Lung cancer is strongly associated with cigarette smoking, nevertheless some never-smokers develop cancer. Immune eradication of cancer cells is dependent on polymorphisms of HLA class I molecules and antigen-processing machinery (APM) components. We have already published highly significant associations of single nucleotide polymorphisms (SNPs) of the *ERAP1* gene with non-small cell lung cancer (NSCLC) in Chinese, but not in Polish populations. However, the smoking status of participants was not known in the previous study. Here, we compared the distribution of APM polymorphic variants in larger cohorts of Polish patients with NSCLC and controls, stratified according to their smoking status. We found significant but opposite associations in never-smokers and in smokers of all tested SNPs (*rs26653, rs2287987, rs30187* and *rs27044*) but one (*rs26618*) in *ERAP1*. No significant associations were seen in other genes. Haplotype analysis indicated that many *ERAP1/2* haplotypes behaved in an opposing way depending on smoking status. Additionally, haplotypic combination of low activity ERAP1 and the lack of an active form of ERAP2 seems to favor the disease in never-smokers. We also revealed interesting associations of some APM polymorphisms with: age at diagnosis (*ERAP1 rs26653*), disease stage (*ERAP1 rs27044*, *PSMB9 rs17587*), overall survival (*ERAP1 rs30187*), and response to chemotherapy (*ERAP1 rs27044*). The results presented here may suggest the important role for ERAP1 in anti-cancer response, different in smokers versus never-smokers, depending to some extent on the presence of ERAP2, and affecting NSCLC clinical course.

26. Unobvious Use of Immunoglobulins

Are immunoglobulins therapy for all diseases – evolution of clinical use

Prof. Anna Pituch-Noworolska[1], Małgorzata Pac[2]

1 – Immunology Ward, University Children Hospital in Cracow, Poland
2 – Department of Immunology, the Children's Memorial Health Institute, Warsaw, Poland

Immunoglobulin replacement therapy (IgRT) began in early 1950's with the first described immunodeficient patients treated by Dr. Bruton with concentrated immune human serum globulin given subcutaneously. However it should be remembered that antibody therapy was used for the first time in the late 19th century.

Immunoglobulin therapy has also been used in other diseases, mostly of autoimmune origin as an immune modulator. An overview of replacement, immunomodulatory and unusual use of immunoglobulin is presented.

Initially immunoglobulin was used to provide passive immunity to patients unable to produce a humoral response to pathogens due to inborn errors of immunity. The other use included pre- and post-exposure prophylaxis to prevent some disease (e.g. hepatitis B, chicken pox et al.).

This therapy has been evaluated in many autoimmune and autoinflammatory diseases. An immune modulatory use of IgG is an approved treatment for immune thrombocytopenia, Kawasaki disease, autoimmune neurological syndromes. Good results of immunoglobulins in these diseases spread the use of this therapy in diseases without established indications or not confirmed clinical effect. Moreover, in diseases with probable autoimmune pathomechanism, immunoglobulins in high dose are used in individual patients with good effect. The latter group includes women with habitual miscarriages and children with symptoms of sudden onset neuropsychiatric syndromes and autoantibodies to the neural structures.

Immunoglobulin therapy is life saving for patients with inborn errors of immunity and impaired antibody production as well as in autoimmune disorders. The variety of available products and modes of administration enable the clinicians to individualize and optimize Ig therapy. Intravenous use of immunoglobulins for autoimmune indications is burdened with the possibility of side effects and the necessity of regular monthly visits to treatment centers. Now, subcutaneous form of immunoglobulins substitution is commonly used with improved life quality of patients.

Immunoglobulins in therapy of immuno-deficiency co-morbidities

Aleksandra Matyja-Bednarczyk

2nd Department of Internal Medicine, Jagiellonian University Medical College, Krakow, Poland
Department of Outpatient Clinic for the Immunological and Hypercoagulable Diseases, The University Hospital in Kraków, Kraków, Poland

Immunoglobulin therapy (IgGRT) represents a lifesaving intervention for many patients with primary immunodefciency (PID). Antibody defects represent approximately half of the well-known PIDs requiring immunoglobulin replacement therapy, most often Common variable immunodefciency disorders (CVID).

CVID constitutes a heterogeneous group of immune defects characterized by hypogammaglobulinemia and failure of specifc antibody production resulting in poor responses to vaccination, increased susceptibility to mainly respiratory infections and autoimmune, infammatory and lymphoproliferative conditions. IgGRTis increasingly recognized as a treatment of a variety of medical conditions, not only for its ability to fght infection as a replacement therapy but also for its antiinfammatory and immunomodulating effects.

IgGRT has been used with varying efcacy, in a number of systemic autoimmune disorders, especially in immune thrombocytopenic purpura (ITP).

I will present the example of patient cases with CVID and successful immunoglobulin therapy in serious co-morbidities.

Substitution of immunoglobulins in incompleted B cell reconstitution after HSCT due to SCID

Anna Szaflarska

Department of Clinical Immunology and Transplantology Jagiellonian University Medical College Children University Hospital, 30-663 Cracow, Wielicka 265

U wielu pacjentów po allogenicznym przeszczepieniu hematopoetycznych komórek macierzystych (HSCT) występują zaburzenia funkcjonowania limfocytów B . Niekiedy konieczna jest substytucja preparatów immunoglobulin, mogą również pojawić się zaburzenia immunoregulacji lub choroby autoimmunizacyjne, co w znacznym stopniu pogarsza jakość życia po HSCT. W wykładzie przedstawiono aktualną wiedzę na temat rekonstytucji poszczególnych subpopulacji limfocytów B u dzieci po HSCT. Omówiono znaczenie źródła komórek macierzystych, rodzaju kondycjonowania oraz wpływ obecności GvHD na proces odbudowy linii limfocyta B. Przekłada się to na częstość infekcji po przeszczepieniu oraz na tworzenie odpowiedzi poszczepiennej , szczególnie przeciwko bakteriom otoczkowym. Odbudowa odporności związanej z limfocytem B może służyć jako wskaźnik sprawności układu immunologicznego po HSCT.

27. Vaccinations in Immunocompromised

BCG-Associated Complications in Primary Immunodeficiency Patients – Thirty-Nine Years of Experience in the Department of Immunology, Children's Memorial Health Institute, Warsaw

Ewa Bernatowska[1], Małgorzata Pac[1], Edyta Heropolitanska-Pliszka[1], Barbara Pietrucha[1], Nel Dąbrowska-Leonik[1], Małgorzata Skomska-Pawliszak[1], Katarzyna Bernat-Sitarz[1], Katarzyna Krzysztopa-Grzybowska[2], Beata Wolska-Kuśnierz[1], Nadia Bohynikova[1], Maria Korzeniewska-Koseła[3], Jean-Laurent Casanova[4], Bożena Mikołuć[5]

1 – Department of Immunology, Children's Memorial Health Institute, Warsaw, Poland
2 – Department of Sera and Vaccines Evaluation, National Institute of Public Health – National Institute of Hygiene, Warsaw, Poland
3 – Department of Tuberculosis Epidemiology and Surveillance, National Tuberculosis and Lung Diseases Research Institute, Warsaw, Poland
4 – Professor, Rockefeller University, New York, USA
5 – Department of Paediatrics, Rheumatology, Immunology and Metabolic Bone Diseases, Medical University of Bialystok, Białystok, Poland

Propose: Our objective was to assess the BCG complication in primary immunodeficiency diseases (PID), group with various inherited disorders and immunological defects, different susceptibility to BCG infection, and reactogenicity to each of BCG substrains.

Methods: The adverse reactions to the locally-produced BCG Moreau vaccine were analyzed in PID patients, diagnosed between 1980 and 2019 in the Department of Immunology, Children's Memorial Health Institute (CMHI), Warsaw.

Results: Significantly fewer disseminated BCG infections (BCGosis), 11 out of 72,15% SCID patients, occurred in comparison with 119 out of 349, 34% ($p = 0.0012$) SCID patients vaccinated with other BCG substrains from other countries analyzed by Beatriz E. Marciano et al. in a retrospective study (Marciano et al. J Allergy Clin Immunol. 2014;133(4):1134–1141).

Results Significantly less disseminated BCG infections (10 out of 52 SCID, 19%) occurred in comparisonin Marciano at all. Significantly fewer death caused by BCGosis, were observed in SCID, only with the NK- phenotype and significantly lower NK cell counts ($p = 0.00031$). A significantly higher number of hematopoietic stem cells transplantations (HSCT) were performed in the CMHI study ($p = 0.00001$). BCGosis was noted, in a total of 6 patients with Mendelian susceptibility to mycobacterial diseases (MSMD). Other PID prone to BCG complication, showed no case of BCGosis, and significantly fewer BCGitis complications in comparison with SCID and MSMD patients ($p < 0.00001$).

Conclusion: The BCG vaccine produced in Poland since 1955 has shown genetic differences in the Moreau substrain, together with a superior safety clinical profile in comparison to the other BCG substrains, with no BCGosis in other than SCID and MSMD patients. Our data confirmed significantly fewer cases of BCGosis and deaths caused by BCG infection in SCID patients , as well as confirmed the protecting role of NK cells.

Vaccinations in children during and after oncological treatment – recommendations of Polish Society of Pediatric Oncology and Hematology

Sylwia Kołtan

Department of Pediatrics, Hematology and Oncology Collegium Medicum Bydgoszcz, Nicolaus Copernicus University Toruń

Infections are one of the biggest threats for patients during and after treatment of neoplastic diseases. Vaccinations are the only effective and safe way to prevent infections. Recommendations regarding their implementation change over time, depending on the current epidemiological situation, access to vaccines, as well as data on the safety of their use in risk groups.

In the lecture are presented the current rules for the implementation of preventive vaccinations according to the recommendations of the of the Polish Society of Hematology and Pediatric Oncology, developed on the basis of the international ECIL 7 guidelines.

In the lecture also are presented information on the current state of knowledge on research on the safety and effectiveness of vaccination against COVID-19 in healthy children and during and after cancer treatment.

Safety and efficacy of vaccination in children with autoimmune neutropenia

Bożena Mikołuć [1], Klaudia Berk [2], Dominika Gładysz[3], Małgorzata Skomska-Pawliszak[3], Małgorzata Pac[3], Ewa Bernatowska[3]

1 – Department of Pediatrics, Rheumatology, Immunology and Metabolic Bone Diseases, Waszyngtona 17, 15-274 Bialystok, Poland
2 – Department of Physiology, Medical University of Bialystok, ul. Mickiewicza 2c, 15-233 Bialystok, Poland,
3 – Clinical Immunology the Children's Memorial Health Institute, al. Dzieci Polskich 20, 04-730 Warsaw, Poland

Aim: The realisation of vaccination schedule in patients with autoimmune neutropenia (AIN) is still opened issue. Thus, in our study, we assessed safety and efficacy of selected vaccines in children with AIN

Materials and methods: Fifty-two children diagnosed with autoimmune neutropenia (M/F ratio 44/56%), aged 0.5 to 16.5 years (mean: 2.56 years) were under the medical care of Department of Immunology at the Children's Memorial Health Institute in Warsaw, Poland. The study group consisted of 8 children with mild (>1000 cells/μl), 26 with moderate (500-1000 cells/μl) and 18 with severe (<500 cells/μl) neutropenia. Vaccine with Hepatitis B, DTaP, MMR ,VZV received 33, 34, 15, and 14 patients, respectively.

The antibodies concentrations after vaccination were measured in the Department of Bacteriology and Clinical Immunology, Children's Memorial Health Institute in Warsaw, and the Department of Sera and Vaccines Evaluation, National Institute of Public Health,Warsaw. The time between vaccination and evaluation of antibodies levels was individual for each patient.

The comparison between the groups was carried out by the Mann–Whitney U-test. For the calculation, a significance level of $p<0.05$ was assumed as being statistically significant. The data was processed by GraphPad Prism.

Results: No adverse events after life and attenuated vaccines were observed. We revealed protective level of antibodies in 99% after VZV and in all patients after MMR vaccination and in 87.9% patients after HBV regardless of the severity of neutropenia. The percentage of seropositive patients for diphtheria was the highest in severe (92%) followed by moderate (75%) and mild (67%) neutropenia. Tetanus protective antibody levels were observed in 50% patients with mild, in 69% with moderate and in 42% with severe neutropenia. Of the 6 patients with mild neutropenia vaccinated against pertussis, only 1 (17%) responded to immunization. In moderate neutropenia, positive antibody level was found in 9 (56%) patients. In severe neutropenia, the number of patients with protective antibody level against pertussis was 7 (58%).

Conclusion: Our data clearly showed that severity of neutropenia has no impact on geometric antibody titers to all vaccine. Live vaccines : MMR and VZV are safety and efficacy in all AIN, despite of neutrophils number. Contrarily, 3 doses of DTaP vaccination do not guarantee protective antibody titers especially against pertussis antigen.

28. Veterinary and Comparative Immunology

Insights on the immune cells – why are we still enchanted?

Paulina Niedźwiedzka-Rystwej

Institute of Biology, University of Szczecin, Poland; Felczaka 3c, 71-412 Szczecin

Some members of diverse immune cells family, despite being known for years, are still surprising because of their roles and properties. Neutrophils are major players of immune responses, controlling bacterial, viral and fungal infections via phagocytosis, degranulation and neutrophil extracellular traps. Lately it is also discussed, that neutrophil counts or the neutrophil-to lymphocyte ratio may be used as a prognostic and predictive biomarker of several types of tumors, on the basis of the fact, that neutrophils reflect a state of host inflammation, which is a hallmark of cancer. Nevertheless, neutrophils' actions need to be strictly regulated to avoid the vascular leakage and excessive release of catalytic proteins. Finally, apoptosis of neutrophils is crucial, as this is the main mechanism of neutrophil death and has a huge impact on viral infections. It is worth mentioning that autophagy of neutrophils is not only involved in differentiation of the cells in bone marrow but also impacts granule formation, degranulation, neutrophil extracellular traps release, cytokine production, bactericidal activity and controlling inflammation.

Adaptive immunity is based on the functioning and network build by lymphocytes, and the number of known lymphocytes subsets is constantly growing. An important issue is the use of T-cells-modulating checkpoint molecules as the most promising therapeutic strategies for cancer. Mainly CD 8+ T lymphocytes traffic into the tumor microenvironment, and exhibit cytotoxicity against tumor cells. At the same time, cytotoxic T cells are pivotal in viral infections, leading (among others) to increased apoptosis.

Autophagy – how intracellular recycling participates in maintaining homeostasis

Małgorzata Gajewska

Department of Physiological Sciences, Institute of Veterinary Medicine, Warsaw University of Life Sciences (SGGW), Nowoursynowska 159b,02-776 Warsaw, Poland

Autophagy is a catabolic process which targets cell constituents to lysosomes for degradation, thus can be described as cellular self-digestion. Macroautophagy is the best characterized type of autophagy in eukaryotic cells. It is based on formation of double membrane vesicles, termed autophagosomes, which engulf long-lived cytoplasmic proteins, damaged organelles, and even invasive pathogens, and transport these cargos to the lysosomes. There, the outer-membrane of the autophagosome fuses with the lysosomal membrane forming an autolysosome, in which the cargo is degraded by the hydrolytic enzymes. Macroautophagy is fundamentally a physiological process that plays indispensable roles in cell restructuring and in ongoing turnover of cellular protein and other macromolecules. For this reason it is regarded as one of the processes playing an essential role in maintaining cellular homeostasis. Moreover, it plays a major role during nutritional deprivation, providing cells with adequate amino acid level to sustain the protein synthesis, as well as to maintain energy supply. Growing body of evidence demonstrate that macroautophagy is critical for cytosolic remodeling during differentiation of some highly specialized cells in mammals, such as adipocytes, mammary epithelial cells; whereas defective autophagy in mammals has been linked to aging, cancer, neurodegenerative diseases, inflammatory diseases, and disorders in liver, muscle, and immune system. Therefore, studies on autophagy and its complex system of intracellular regulation remains a hot topic for researchers specializing in cell biology, developmental biology, physiology, pathophysiology and medicine worldwide.

The possibilities of cancer immunotherapy in veterinary medicine

Iwona Szopa, Kinga Majchrzak-Kuligowska

Warsaw University of Life Sciences, Institute of Veterinary Medicine, Department of Physiological Sciences, Warsaw, Poland

Cancer immunotherapy is a novel method of neoplasms treatment that gives hope to the patients with advanced solid tumors. Human hematological malignancies and melanoma have been already successfully treated with various types of immunotherapy such as adoptive cell transfer, cancer vaccines or immune checkpoint blockade. Due to the similarities between canine and human antitumor immunity, a parallel approach can be applied in the veterinary oncology. However, except of canine oral melanoma vaccine, the other immunotherapy types are currently under development.

The adoptive cell transfer (ACT) therapy has demonstrated great promise in canine clinical trials. ACT treatment involves the infusion of tumor-specific T lymphocytes into the circulatory system of cancer patients. T cells are isolated from patients' peripheral blood and genetically modified ex vivo to express the chimeric antigen receptors (CARs), which are the synthetic receptors recognizing tumor-associated antigens. Thus, CAR-T cells are able to precisely identify and effectively eradicate cancer cells.

Recently, several studies assessing efficiency and safety of cellular immunotherapy on canine non-Hodgkin lymphoma, B cell lymphoma and osteosarcoma have been performed. They showed that ACT using CAR-T cells is feasible and safe in dogs and can result in durable remissions and prolonged survival of canine patients. Furthermore, adoptive NK cells transfer in combination with radiotherapy has shown to be effective treatment for dogs with osteosarcoma.

Research conducted by our team focuses on the subpopulation of canine Th17 lymphocytes in order to improve immunotherapy protocols for humans. Th17 cells are the subset of interleukin 17-producing T cells characterized by plasticity and self-renewal ability. They showed remarkable effectiveness in melanoma eradication on ACT mouse model. The aim of our study is to exploit and improve anticancer activity of canine Th17 cells by modification of intrinsic signaling pathways in vitro.

In summary, studies on the pet dogs, being the patients of veterinary clinics, are important in extending the range of anticancer therapies offered for animals and valuable tool for comparative oncology.

Our project has been carried out within the First TEAM programme of the Foundation for Polish Science co-financed by European Union under the European Regional Development Fund (POIR.04.04.00-00-3EE9/17-00).

Properties of antimicrobial peptides and their potential applications in veterinary medicine

Joanna Sylwia Wessely-Szponder

Department of Pathophysiology, Institute of Preclinical Veterinary Sciences, Faculty of Veterinary Medicine, University of Life Sciences in Lublin

Antimicrobial peptides (AMPs), also known as immunomodulatory or host defence peptides (IHDP/HDP), are of growing interest due to their properties and potential for clinical applications. Within them neutrophil-derived antimicrobial peptides are an important and effective component of innate immunity and possible alternative to conventional antibiotics. In addition to the main antimicrobial activity, especially against gram-positive and gram-negative bacteria, fungi and viruses, AMPs also have an immunomodulatory effect, regulating the course and severity of the inflammatory reaction. This immune activation is closely related to the type of cells, environmental stimulators, interactions with various cell receptors, and the concentration of the peptides. The immunomodulatory properties of AMPs include; reduction in the levels of proinflammatory cytokines produced in response to pathogens, modulation of the expression of chemokines, generation of reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI), stimulation of angiogenesis, enhanced wound healing, leukocyte activation, and macrophage differentiation.

As a part of the One Health approach, animal research will make the way for potential therapeutic success of AMPs as an alternative to antibiotics in veterinary medicine. AMPs can be used as a vaccine adjuvant, as an adjunct to conventional antibiotics, or directly as antimicrobials. Interactions with biomaterials to prevent infections associated with the implantation of orthopaedic implants were also studied on animal models. Previous study indicated the potential for the use AMPs as stimulator of bone regeneration with antimicrobial properties. Therefore, our research team conducted research on the possible modulation of the inflammatory response during surgery. We estimated that autologous AMPs can stimulate neutrophil activity, whereas heterologous AMPs can suppress it. Macrophages after stimulation with AMPs acquire partially proinflammatory and partially antiinflammatory features and they can generate different types of inflammatory mediators. This intermediate phenotype seems to be a good solution for modulating of inflammatory response. After clinical trials the obtained results may be useful during surgical procedures by modifying the white blood cell system.

Acute phase proteins in rabbits in acute viral infection, on the example of GI.1a-RHDVa virus (rabbit haemorrhagic disease virus)

Łukasz Baraniecki[1], Beata Tokarz-Deptuła[1], Andrzej Pępiak[2], Wiesław Deptuła[3]

1 – Institute of Biology, University of Szczecin
2 – Dr Andrzej Pępiak Domestic Animal Clinic, West Pomeranian Veterinary Education Center of Szczecin
3 – Institute of Veterinary Medicine, Faculaty of Biological and Vaterinary Sciences, Nicolaus Copernicus University in Toruń

The immune response in acute viral infections, e.g. infections caused by hemorrhagic fever viruses, is related to the elements that create reactivity of the macroorganism, appearing in the first hours after infection. Among these elements are acute phase proteins (APPs), which so far have been rarely studied in rabbits, while in those animals infected with the highly infectious GI.1a-RHDVa virus causing rapid and acute disease, have not been conducted before. The experiment was carried out on 40 healthy Polish rabbits, which were experimentally infected with GI.1a-RHDVa virus – Dutch strain NL-2 with positive haemagglutination, which caused high mortality and was characterized by an acute course of the disease lasting basically up to 24-72 hours. In the experiment, three groups of 10 infected animals were created and the fourth group of 10 control animals. In infected animals (groups I-III) the concentration of C-reactive protein (CRP) – group I, serum amyloid A (SAA) – group II and haptoglobin (Hp) – group III – was determined. APPs concentration in the blood of groups I-IV was chacked every 12 hours in the first two days (0-48h), and every 4 hours between 48-60 hours, using the USCN Life Science ELISA test. Due to the death of infected rabbits, the CRP protein was determined up to 60 hours (group I), the SAA protein up to 48 hours (group II), and the Hp protein up to 52 hours (group III). It was found that statistically significant differences in CRP protein values were obtained at 36, 48 and 52 hours, SAA proteins at 12, 24 and 36 hours, and Hp proteins at 12, 24, 36 and 48 hours after infection of the animals. The obtained picture of changes in the three examined APPs shows that they are important indicators in rabbits in this infection, and the picture of these changes proves that not only the CRP protein plays an important role in these animals, as it has been assumed so far, but also proteins SAA and Hp play key role.

Initial investigation on NP-5 (corticostatin-6) defensin expression in liver of healthy and *Lagovirus* europaeus infected rabbits

Hrynkiewicz R.[1], Bębnowska D.[1], Niedźwiedzka-Rystwej P.[1]

1 – Institute of Biology, University of Szczecin, Felczaka 3c, 71-412, Szczecin, Poland

Antimicrobial peptides (AMP) are very important element of innate immunity, and the mechanisms within it constitute the body's primary line of defense in contact with infectious agents. One of the representatives of AMP are defensins that play a key role in viral, bacterial and fungal infections. For rabbits, information on defensins remains fairly limited. It is certain that the mechanisms of innate immunity are of key importance in the pathogenesis of rabbit haemorrhagic disease caused by *Lagovirus europaeus*, but the studies available so far have not considered the defensin characteristics in viral infections. For these reasons, the main objective of this study was to verify the presence of defensin NP-5 (corticostatin-6) in the livers of healthy and RHDV (*Lagovirus europaeus*) infected rabbits, as a first step to further analysis of the involvement of these proteins in the course of this viral disease. Qualitative analyzes were performed using the Real-Time PCR method with the use of specific primers. As a result, the presence of NP-5 defensin was confirmed with high accuracy in all tested samples. This is the first study to investigate the presence of defensins in the livers of healthy and *Lagovirus europaeus* infected rabbits and further experiments are needed.

Comparison of the antimicrobial activities and the survival rates of the greater wax moth *Galleria mellonella* infected with *Pseudomonas entomophila* via oral and intrahemocoelic routes

Jakub Kordaczuk, Michał Sułek, Iwona Wojda

Department of Immunobiology, Institute of Biological Sciences, Maria Curie-Sklodowska University, Lublin, Poland

Pseudomonas entomophila (Pe) is the only entomopathogenic *Pseudomonas* strain that is able to infect insects orally. The complete genome sequence of this strain identified a number of potential virulence factors. So far, the research focused on understanding the mechanism of the pathogen-host interaction has been conducted on the fruit fly *Drosophila melanogaster*. However, little is known about its activity against other insect species. In our work, we investigated the entomopathogenic properties of Pe on a Lepidoptera representative – the greater wax moth *Galleria mellonella*, which is widely used as a model organism in studies of innate immunity and host-pathogen interactions.

We studied the differences in the survival rate of the greater wax moth *G. mellonella* larvae after oral infection with Pe and after injection of a specific dose of bacteria to the insect's hemocoel. The latter can happen in a natural environment after wounding the cuticle. We observed significant differences in the insect survival rate depending on the dose of bacteria and the type of infection. The hemolymph of the infected caterpillars was collected at specific time points and tested for antimicrobial activity. In the case of the intrahemocoelic injection, the antimicrobial activity detected in larval hemolymph was higher after application of the higher dose of Pe and lower after infection with the lower dose. Surprisingly, this dependency was not maintained in the hemolymph of caterpillars infected orally. In the case of oral infection, the antimicrobial activity of the hemolymph was very high after the low dose of bacteria and significantly lower after infection with the higher dose. We believe that focusing on both oral and injection modes of infection will help in our understanding of immune processes occurring during infection of the studied host.

Acknowledgement: *Pseudomonas entomophila* is a kind gift from dr Frideric Boccard (France), whose group isolated this strain in Guadeloupe.

Regulatory role of IFN-λ in canine mammary cancer cells survival and their ability to migrate *in vitro*

Rafał Pingwara, Iwona M. Szopa, Daria Kosmala, Natalia Woźniak, Kinga Majchrzak-Kuligowska, Arkadiusz Orzechowski, Joanna Mucha

Department of Phsyiological Sciences, Institute of Veterinary Medicine, Warsaw University of Life Sciences – SGGW

Mammary gland tumors are the most common tumors among non-castrated female dogs. IFN-λ is one of the factors present in the canine mammary tumor. According to previous reports on human and murine cell lines, IFN-λ induces apoptosis and inhibits the proliferation of cancer cells. However, IFN-λ may enhance the process of tumor metastasis. The study aimed to determine the effect of IFN-λ on the survival of canine mammary tumor cells (CMTc) and their migration ability.

The study was conducted on three CMTc lines: (P114, U27, U309) cultured under standard conditions. 24 hours prior to the experiment growth medium (RPMI+10%FBS) was replaced with control medium (RPMI+2%BSA), subsequently, CMTc were treated with human recombinant IFN-λ1 or -λ2. Cell respiration (MTT), proliferation (crystal violet), and apoptosis (annexin V) were tested to assess cells survival. Cell migration was examined in a scratch test, expression of extracellular matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) genes was examined by RT-qPCR. The expression of IL-10Rβ and IL-28RA subunits of IFN-λ receptor was determined by the Western Blot.

Expression of IL-28RA was found in all tested cell lines, while the IL-10Rβ expression was detected only in CMT-U27 and P114. No effect of IFN-λ on cell survival was observed, except for P114 cells treated with IFN-λ1 at concentrations of 500 and 1000 ng/ml. 48-hours IFN-λ treatment significantly increased the wound closing in P114 cells in comparison to control, while reduced this process by U27 cells. Whereas no effect of IFN-λ on U309 cells motility was observed. There was statistically significant increase in *mmp-3* and *mmp-9* expression and decrease in the levels of their inhibitors (*timp-1* and *timp-2* in P114 cells treated with IFN-λ for 24 hours. Statistically significant increase in *timp-1* expression in U27 cells treated with cytokines was also shown.

The obtained results indicate that IFN-λ have cell line-dependent effect on CMTc migration abilities, however, do not participate in the regulation of cell survival. IFN-λ modulate CMTc migration via regulation of expression of the genes controlling extracellular matrix degradation, yet this mechanism requires further research.

The research was founded by National Science Centre (2016/23/D/NZ5/03276).

Immune response of the greater wax moth *Galleria mellonella* (Lepidoptera) after injection with *Pseudomonas entomophila*

Michał Sułek[1], Jakub Kordaczuk[1], Iwona Wojda[1]

1 – Department of Immunobiology, Institute of Biological Sciences, Maria Curie-Sklodowska University, Lublin, Poland

The relatively newly discovered *Pseudomonas entomophila* (Pe) is unique among *Pseudomonas* species, as it exhibits strong enthomopathogenic properties against *Drosophila melanogaster* and against other insects. The fast colonization of the insect's gut by Pe leads to insect's death. In a natural environment, the bacteria can also reach the hemocoel through wounded cuticle. These properties make Pe a perfect model to study the insect's immune reactions and pathogen-host interactions. Despite the prominent attributes of Pe as a natural insect pathogen, the knowledge of the immune response of infected insects is limited. The greater wax moth *Galleria mellonella* (order: Lepidoptera) is a widely used model organism to study pathogen-host interactions. The easy breeding, short generation time, and sequenced genome makes *Galleria mellonella* an excellent object to study immune mechanisms. What is more, the greater wax moth is a perfect source of insect AMPs (antimicrobial peptides), which are small (2-10kDa) molecules with a broad spectrum of activity against microorganisms.

In our study, we examined the survival rate and some immune response parameters of *Galleria mellonella* infected *via* injection of *Pseudomonas entomophila* directly into insect's hemocel. We collected the hemolymph of infected larvae at specific time points and compared the activity of two major enzymes engaged in insect's humoral defense: phenoloxidase and lysozyme. Furthermore, we analyzed the antimicrobial activity of the hemolymph using the bioautography assay and compared the protein profile obtained with Tris-Tricine SDS-PAGE. We observed significant changes in enzymatic activity and antimicrobial properties detected in the hemolymph of infected insects compared to the control groups. Furthermore, Tris-Tricine SDS-PAGE assays revealed abundance of inducible peptides in the hemolymph samples obtained from infected larvae at the peak of ongoing infection. Our research provides the new information about the overall immune response of the Lepidoptera representative *Galleria mellonella* to highly entomopathogenic *Pseudomonas entomophila*. Ultimately, inducible peptide-rich hemolymph from infected caterpillars is a potential source of new proteins or antimicrobial peptides, which may find application in future medicine.

Acknowledgement: *Pseudomonas entomophila* is a kind gift from dr Frideric Boccard (France), whose group isolated this strain in Guadeloupe.

Programming of CD4+ T lymphocytes toward Th17 phenotype – *in vitro* study on companion dog model

I.M. Szopa, R. Pingwara, M. Granica, K. Majchrzak-Kuligowska

Warsaw University of Life Sciences, Institute of Veterinary Medicine, Department of Physiological Sciences, Warsaw, Poland

Th17 lymphocytes are characterized by their ability to produce IL-17 and express retinoic acid-related orphan receptor (RORγt). Being resistant to apoptosis and exhibiting stem cell-like properties, they maintain a potent antitumor activity after long-term *ex vivo* expansion. Th17 cells have been shown to efficiently eradicate large established melanoma tumors upon adoptive cell transfer (ACT) therapy on mouse model. We have further developed research in this field by using domestic dog model (*Canis lupus familiaris*), which is attractive and useful for comparative medicine. Unlike transplantable xenograft rodent models, canine tumors occur spontaneously and share similar genetics, prognosis factors and clinical outcomes with human tumors.

The aim of this study was to determine activation and expansion protocols for canine $CD4^+$ T lymphocytes as well as their programing toward Th17 phenotype. Furthermore, we modified intrinsic signaling pathways of canine T cells in order to support Th17 differentiation.

We isolated $CD4^+$ T lymphocytes from peripheral blood of domestic dogs and activated them using either Concanavalin A (natural mitogen) or epoxylated magnetic beads coated with anti-canine CD3 and CD28 antibodies. For type 17 polarization canine IL-1β, IL-6, TGF-β, anti-IL-4 and anti-IFN-γ antibodies were used. Moreover, the effect of indomethacin (blocker of β-catenin expression) on the Th17 programing was examined.

Our research showed increased CD25 expression and IL-2 production by canine $CD4^+$ T cells as well as morphological changes during blast transformation upon activation using epoxylated magnetic beads. Moreover, we successfully programmed canine Th17 lymphocytes, which have exhibited higher RORγt expression and have released significant amount of IL-17 in comparison to non-stimulated cells. Our study revealed that administration of indomethacin further enhanced IL-17 production (up to 55%) and up-regulated expression of Th17 cells-associated genes.

Overall our research determined the optimal conditions for canine Th17 cells expansion for further immunological assessment. Moreover this study paved the way for tumor-bearing dog studies of ACT with Th17 cells, which may benefit and facilitate design of human clinical trials.

The project has been carried out within the First TEAM programme of the Foundation for Polish Science co-financed by European Union under the European Regional Development Fund (POIR.04.04.00-00-3EE9/17-00).