INTERNATIONAL MOLECULAR IMMUNOLOGY & IMMUNOGENETICS CONGRESS IV (MIMIC IV)
27-29 April 2019
Mete Cengiz Congress and Culture Center - Bursa / TURKEY

Congress President
Haluk Barbaros Oral

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Final Program & Abstracts
www.mimic2019.org
INTERNATIONAL MOLECULAR IMMUNOLOGY & IMMUNOGENETICS CONGRESS IV (MIMIC IV)

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Final Program & Abstracts
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INVITATION

Dear Colleagues and Friends,

It is with great pleasure that we welcome you on behalf of the Turkish Society of Immunology (TSI) to the International Molecular Immunology & Immunogenetics Congress IV (MIMIC IV) in the mountainside beautiful city of Bursa in Turkey from April 27-29, 2019.

Held every alternating year since 2012, MIMIC IV promises to continue this tradition by staging a truly memorable scientific event for all non-clinical and clinical attendees. MIMIC IV will cover all fields of modern immunology, including innate, memory and regulation of T and B cells. Recent trends and emerging topics on molecular and applied immunology as well as immunogenetics will be discussed. As always, MIMIC Organizers put special effort to unite internationally renowned scientists with new faculty and young researchers in a friendly and relaxed environment. Aiming to foster the exchange of ideas along with facilitating collaborations in this MIMIC IV we invite an exceptional group of scientists to discuss their findings and discoveries. We believe that MIMIC IV will be an excellent platform to facilitate this mutual goal while tackling challenges that arise from connecting fundamental and translational science.

This year, the organizing committee has made special efforts to support early-stage scientists/researchers via several grants and awards covering registration, accommodation, and part of travel expenses. Therefore, young researchers are particularly encouraged to participate in this vibrant and exciting congress.

We are looking forward to seeing you in Bursa for this unique and stimulating event.

Sincerely,

Prof. H. Barbaros ORAL

Congress President
COMMITTEES

SCIENTIFIC COMMITTEE

Halis Akalin (TUR)
Cezmi Akdis (SUI)
Mubeccel Akdis (SUI)
Shizuo Akira (JPN)
Sefik Sanal Alkan (SUI)
Arzu Aral (TUR)
Carolina Arancibia (UK)
Moshe Arditi (USA)
Semir Beyaz (USA)
Yildiz Camcioglu (TUR)
Cevayir Coban (JPN)
Gulderen Yanikkaya-Demirel (TUR)
Gunnur Deniz (TUR)
Guher Saruhan-Direskeneli (TUR)
Pablo Engel (ESP)
Gunes Esendagli (TUR)
Dicle Guc (TUR)
Ihsan Gursel (TUR)
Mayda Gursel (TUR)
Ken J. Ishii (JPN)
Elif Karakoc (TUR)
Micheal Lohoff (GER)
Barbaros Oral (TUR)
Andreas Radbruch (GER)
Hannes Stockinger (AUT)
Tolga Sutlu (TUR)
Andrey Tchorbanov (BUL)
Duygu Ucar (USA)
Derya Unutmaz (USA)
Willem Van De Veen (SUI)
Gerhard Wingender (TUR)
Masahiro Yamamoto (JPN)
Ayca Sayi Yazgan (TUR)
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<td>Haluk Barbaros Oral</td>
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<td>Ihsan Gursel</td>
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<td>Gunnur Deniz</td>
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<td>Guher Saruhan Direskeneli</td>
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<th>LOCAL ORGANIZING COMMITTEE</th>
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<tr>
<td>Elif Ardahanli</td>
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<td>Ferah Budak</td>
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<td>Pinar Ellergezen</td>
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<td>Ertan Kanbur</td>
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<td>Mert Karaca</td>
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<td>Mehmet Karacay</td>
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<td>Muhammed Ali Kizmaz</td>
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<td>Mehrdad Pashazadeh</td>
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<td>Izel Yilmaz</td>
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*(in alphabetical order by last name)*
## SCIENTIFIC PROGRAM

**Saturday, April 27th 2019**

### HALL A

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<td>Barbaros ORAL, Gunnur DENIZ</td>
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<td>12:00 - 13:00</td>
<td><strong>REGNASE-1, AN ENDORIBONUCLEASE INVOLVED IN INFLAMMATION AND IMMUNE RESPONSES</strong></td>
<td>SHIZUO AKIRA (JPN)</td>
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<td>13:00 - 14:30</td>
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<td>SATTELITE SYMPOSIUM</td>
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<td><strong>SESSION 1</strong></td>
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<td>14:30 - 15:00</td>
<td>INNATE IMMUNE SYSTEM AND VACCINE ADJUVANTS</td>
<td>Gunnur DENIZ, Derya UNUTMAZ</td>
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<td>14:30 - 15:00</td>
<td><strong>DEEP LEARNING OF VACCINE BY MACHINE AND IMMUNOLOGY</strong></td>
<td>KEN J. ISHII (JPN)</td>
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<td>15:00 - 15:30</td>
<td><strong>PREVENTIVE AND THERAPEUTIC LEISHMANIA VACCINE DEVELOPMENT</strong></td>
<td>MAYDA GURSEL (TUR)</td>
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<td>15:00 - 15:30</td>
<td><strong>NEW AGE VACCINATION USING ASC SPECKS AS A CARRIER/ADJUVANT</strong></td>
<td>AYLIN ALKAN (TUR)</td>
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<td>15:45 - 16:00</td>
<td><strong>IMMUNE MODULATORY THERAPEUTIC APPLICATIONS OF PEDIOCOCCUS PENTOSACEUS DERIVED MEMBRANE VESICLES</strong></td>
<td>ESIN ALPDUNDAR BULUT (TUR)</td>
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<td>16:30 - 17:00</td>
<td>ALLERGEN SPECIFIC B-CELL RESPONSES IN A HUMAN MODEL OF HIGH-DOSE ALLERGEN EXPOSURE</td>
<td>Willem van de Veen (SUI)</td>
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<td>17:00 - 17:30</td>
<td>NOVEL B-CELL SUBSETS AND IMMUNE TOLERANCE</td>
<td>Mubeccel Akdis (SUI)</td>
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<td>17:30 - 17:45</td>
<td>IN Variant NATURAL KILLER T (iNKT) CELLS ARE NOT ESSENTIAL FOR MURINE NEUTROPHILIC ASTHMA PATHOGENESIS</td>
<td>Muge Ozkan (TUR)</td>
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<td>17:45 - 18:00</td>
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<td>Nikola Ralchev (BUL)</td>
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<td>18:00 - 18:30</td>
<td>THE ROLE OF INTERFERON REGULATORY FACTORS DURING T-CELL SUBSET DIFFERENTIATION</td>
<td>Michael Lohoff (GER)</td>
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<td>WELCOME COCKTAIL &amp; POSTER SESSIONS</td>
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<td>SPEAKERS' DINNER</td>
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<td>Ihsan Gursel (TUR)</td>
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<td>08:00 - 08:30</td>
<td>ACCURATE MOLECULAR DIAGNOSIS TRANSLATES INTO BETTER TREATMENTS IN PIDs: A MARMARA EXPERIENCE</td>
<td>Elif Karakoc (TUR)</td>
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<tr>
<td>08:30 - 09:00</td>
<td>BOTTLENECKS AND SOLUTIONS IN ANTIGEN-SPECIFIC ADOPTIVE IMMUNOTHERAPY OF CANCER</td>
<td>Tolga Sutlu (TUR)</td>
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<td>Pattraporn Satitsuksanoa (SUI)</td>
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<td>09:15 - 09:30</td>
<td>AN IMMUNOSUPPRESSIVE OLIGODEOXYNUCLEOTIDE CONTAINING TELOMERIC TTAGGG MOTIFS ACT THROUGH PI3K/MTOR PATHWAY TO MODULATE IMMUNE CELL METABOLISM</td>
<td>GIZEM KILIC (TUR)</td>
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<td>THEMES ON THE FUNCTION AND PLASTICITY OF iNKT CELL SUBSETS</td>
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<td>11:00 - 11:30</td>
<td>REVISITING HELPER T CELL ACTIVATION IN CANCER</td>
<td>THE 10TH SYMPHONY PLAYED BY THE IMMUNE ORCHESTRA</td>
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<td>12:00 - 12:30</td>
<td>NANOSCALING THE ORGANIZATION AND SIGNALING OF THE T CELL ANTIGEN RECEPTOR FOR IMMUNOTHERAPY</td>
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<td>12:30 - 12:45</td>
<td>DIETARY AND EPIGENETIC REGULATION OF CANCER AND IMMUNITY</td>
<td>A SYSTEMATIC COMPARISON OF DIFFERENT CHIMERIC ANTIGEN RECEPTOR (CAR) DESIGNS FOR RETARGETING OF NK-92 CELLS AGAINST TUMOR ANTIGENS</td>
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<td>EFFECTS OF CANCER ASSOCIATED FIBROBLASTS ON MONOCYTES AND MACROPHAGES IN BREAST CANCER</td>
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<td>13:00 - 14:00</td>
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<td>SESSION 6</td>
<td>IMMUNE DYSFUNCTION AND MICROBIOME</td>
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<td>DECIPHERING THE HUMAN IMMUNE LANDSCAPE AND MICROBIOTA FOR PRECISION MEDICINE</td>
<td>DECIPHERING THE HUMAN IMMUNE LANDSCAPE AND MICROBIOTA FOR PRECISION MEDICINE</td>
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<td>14:30 - 15:00</td>
<td>ROLE OF MICROBIOME, INCREASED GUT PERMEABILITY AND SECRETORY IgA IN KAWASAKI DISEASE VASCULITIS MOSHE ARDITI (USA)</td>
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<td>15:00 - 15:15</td>
<td>IgE PRODUCTION IN CHRONIC GRANULOMATOUS DISEASE RELATED TO FUNGAL ANTIGENIC STIMULATION ISMAIL OGULUR (TUR)</td>
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<td>15:15 - 15:30</td>
<td>SMALL CELL LUNG CANCER STEM CELLS SHOW ADAPTIVE RESISTANCE THROUGH EFFECTIVELY INDUCING T CELL ACTIVATION AND MODULATING THE EXPRESSION OF CO-INHIBITORY RECEPTORS M. ALPER KURSUNEL (TUR)</td>
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<td></td>
<td>GENETIC MANIPULATION OF IMMUNE SYSTEM</td>
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<td>Chairs: Arzu ARAL, Carolina ARANCIBIA</td>
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<td>15:30 - 16:00</td>
<td>CD48 VIRAL HOMOLOGS AS NOVEL MANIPULATORS OF IMMUNITY PABLO ENGEL (ESP)</td>
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<td>16:00 - 16:30</td>
<td>GENOMIC SIGNATURES OF HUMAN IMMUNE SYSTEM AGING DUYGU UCAR (USA)</td>
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<td>16:30 - 16:45</td>
<td>MOLECULAR MECHANISM FOR M1 BIAS OF ABCG1-DEFICIENT MACROPHAGES MELTEM ALTUNAY (TUR)</td>
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<td>16:45 - 17:00</td>
<td>REJECTION IN AN IMMUNE PRIVILEGED TISSUE: CHARACTERISTICS OF IMMUNE CHECKPOINTS AND LYMPHOCYTE SUBSETS IN CORNEAL REJECTION FEHIM ESEN (TUR)</td>
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<td>Chairs: Moshe ARDITI, Duygu UCAR</td>
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<td>HOST-MICROBIAL INTERACTIONS IN IBD AND OBESITY CAROLINA ARANCIBIA (UK)</td>
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<td>18:00 - 18:15</td>
<td>GENETIC MODIFICATION STRATEGIES OF THE DENDRITIC CELLS FOR THE INDUCTION OF IMMUNE TOLERANCE IN COLLAGEN-INDUCED ARTHRITIS (CIA) MOUSE MODEL IZEL YILMAZ (TUR)</td>
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<tr>
<td>18:15 - 18:30</td>
<td>SUPPRESSION OF AUTOREACTIVE T AND B LYMPHOCYTES BY ANTI-ANNEXIN A1 ANTIBODY IN HUMANIZED MURINE MODEL OF SYSTEMIC LUPUS ERYTHEMATOSUS SILVIYA BRADYANOVA (BUL)</td>
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<td>MAINTENANCE OF IMMUNOLOGICAL MEMORY ANDREAS RADBRUCH (GER-EFIS PRESIDENT)</td>
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<td>AYCA SAYI YAZGAN (TUR)</td>
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<td>MASAHIRO YAMAMOTO (JPN)</td>
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<td>YAVUZ F. YAZICIOGLU (TUR)</td>
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<td>ANDREY TCHORBANOV (BUL)</td>
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<td>AHMET HAZINI (GER)</td>
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<td>12:45 - 13:00</td>
<td>HUMAN UMBILICAL CORD TISSUE MESENCHYMAL STEM CELL (UCX®) EXOSOMES AS</td>
<td>OZLEM BULUT (TUR)</td>
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<td>CEZMI AKDIS (SUI)</td>
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<td>13:45 - 14:00</td>
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ORAL ABSTRACTS
GENETIC MODIFICATION STRATEGIES OF THE DENDRITIC CELLS FOR THE INDUCTION OF IMMUNE TOLERANCE IN COLLAGEN-INDUCED ARTHRITIS (CIA) MOUSE MODEL

Izel Yilmaz, Mehmet Karacay, Gokcen Guvenc, Didem Unsal, Elif Uz, Arzu Yilmaztepe Oral, Ferah Budak, Figen Ersoy, Tolga Sutlu, Murat Yalcin, Ahmet Akkoc, Haluk Barbaros Oral

Department Of Medical Immunology, Institute Of Health Sciences, Bursa Uludag University, Bursa, Turkey
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Faculty Of Engineering And Natural Sciences, Sabanci University, Istanbul, Turkey
Department Of Molecular Biology & Genetics, Faculty Of Arts & Sciences, Bursa Uludag University, Bursa, Turkey
Department Of Medical Biochemistry, Faculty Of Medicine, Bursa Uludag University, Bursa, Turkey
Department Of Immunology, Faculty Of Medicine, Bursa Uludag University, Bursa, Turkey
Department Of Physiology, Faculty Of Veterinary, Bursa Uludag University, Bursa, Turkey
Department Of Pathology, Faculty Of Veterinary, Bursa Uludag University, Bursa, Turkey

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T lymphocyte activation has an important role in RA pathogenesis. Co-stimulatory signal blockage and tryptophan catabolism have key role in the induction of tolerance. In this study, to inhibit the activation of T lymphocytes in experimental arthritis, tolerogenic dendritic cells (tolDCs) were aimed to be obtained by the genetic modifications of bone marrow-derived dendritic cells (BM-DCs).

One of the genetic modification strategies aims B7 co-stimulatory molecules expression down-regulation in BM-DCs with a gene construct encoding a modified cytotoxic T lymphocyte antigen 4 molecule (CTLA4-KDEL) which targets to the endoplasmic reticulum (ER). The other genetic modification aims continually expression of indoleamine-2,3-dioxygenase (IDO) from BM-DCs which may cause tryptophan catabolism in the synovium.

Mouse CTLA4 cDNA (pCMV/mCTLA4) and human IDO cDNA mammalian expression plasmids (pCMV/hIDO) were commercially provided from Sino Biologicals (China). The mCTLA4 gene was cloned into pCMV/myc/ER (Invitrogen, Life Technologies, USA) to obtain CTLA4-KDEL gene construct. For lentivirus production, plasmids subcloned into LeGo-iG2 (AddGene, USA). BM-DCs were obtained from BALB/c mice, cultured and lentiviral transfected with CTLA4-KDEL and hIDO incubated for 48 hours. Flow cytometric analysis was performed using mouse monoclonal antibodies against CD80 and CD86 (Tonbo Biosciences, United Kingdom) and appropriate isotype controls. As in vivo studies, modified BM-DCs were transferred intraarticularly 3 weeks after collagen-induced rheumatoid arthritis (CIA) mouse model and followed up for 4 weeks. The therapeutic effects evaluated by considering ankle circumference, clinical and histopathological scoring of mice, CD4+ T cell and CD68+ synovial macrophages ratios comparing to control treatment.

It was observed that CD80/86 expression on the surface of BM-DCs significantly downregulated as tolDCs. Moreover, in vivo studies showed that ankle circumference, clinical and histological scores of mice and ratios of CD4+ T cells and CD68+ synovial macrophages significantly decreased in CIA mice which are treated with genetically modified BM-DCs.
The results from this study demonstrate that genetically modified BM-DCs have exciting potential to lead a new approach for the treatment of RA and other autoimmune diseases.

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SUPPRESSION OF AUTOACTIVE T AND B LYMPHOCYTES BY ANTI-ANNEXIN A1 ANTIBODY IN HUMANIZED MURINE MODEL OF SYSTEMIC LUPUS ERYTHEMATOSUS

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Introduction: Systemic lupus erythematosus (SLE) is a complex autoimmune disease that affects multiple organs and systems. Typical for SLE are many abnormalities such as generation of autoantibodies against self-nuclear antigens, glomerulonephritis, vasculitis, inadequate immune complex clearance by phagocytes and shifting toward Th2 response. Annexin A1 (ANX A1) is a 37 kDa protein and a member of the superfamily of annexin proteins. Alike other members of this superfamily, aided by Ca2+ ANX A1 binds to acidic phospholipids. The most studied roles of this protein are the inhibition of phospholipase A2 (PLA2) activity and its role in the resolution of inflammation.

Purpose: We hypothesize that it may be possible to down-regulate the activity of autoreactive T and B cells from SLE patients in humanized immunodeficient mouse model by treating them with an antibody against ANX A1.

Method: The immunomodulatory activity of the therapeutic antibodies was tested in vitro and in vivo. We humanized the NOD-SCID mice with human peripheral blood mononuclear cells (PBMCs) either from healthy donors or SLE patients. FACS was used for the detection of ANXA1 expression, activation markers and apoptosis assay. The levels of anti-dsDNA antibodies in the mice sera and the number of human dsDNA producing plasma cells were quantified by ELISA, ELISpot and protein array.

Results: The cultivation of PBMCs from lupus patients in the presence of the anti-ANX A1 antibody decreases the number of IgG anti-dsDNA antibody-secreting plasma cells, influenced T cell proliferation and activation markets, and induced B and T cell apoptosis. We employed a humanized model of SLE by transferring PBMCs from lupus patients to immunodeficient NOD-SCID mice. The humanized animals showed presence of autoantibodies, proteinuria, and immunoglobulin deposition in the renal glomeruli. The treatment reduced the levels of autoantibodies to several autoantigens, lupus-associated cytokines and disease symptoms.

Discussion: The fine tuning of T cell activation in dependence of ANX A1 quantity is additional mechanism for modulation of the adaptive immunity, being a bridge and a regulator of the innate immunity functions. The presented humanized NSG model of SLE explore a novel approach for suppression of autoreactive B and T cell cooperation during the disease progression. Using low amount of a monoclonal antibody against ANX A1 we restricted disease symptoms progression and pathological kidney damages, thus providing insight on a new potential therapy for human SLE.
TARGETED ELIMINATION OF DER P 1-SPECIFIC B CELLS IN HUMANIZED SCID MOUSE AND MURINE HDM ALLERGY MODELS

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Introduction: Der p1 is allergenic molecule of Dermatophagoides pteronyssinus (Dpt) which causes house dust mite (HDM) allergy. The pathological Der p1-specific B cells produce allergen-specific IgE antibodies that mediate most of the hypersensitivity allergic reactions. It may be possible to influence Der p1-specific B cells in humanized mouse model of HDM allergy by administration of chimeric molecule, containing the 3D9 monoclonal antibody which targets the B cell inhibitory complement receptor type 1 (CR1) coupled to a B and a T cell epitope-carrying peptides from the Der p1 allergen. In order to observe more features of the allergy and the therapeutic effect of chimeric molecules we established a murine HDM allergy model. The murine chimeric molecule contain a monoclonal antibody against the inhibitory B-cell receptor FcγRIIb and the same antigenic peptide from the Der p1 allergen.

Purpose: Co-crosslinking of the immunoglobulin receptors and the inhibitory B-cell receptors by the chimeric molecules is expected to deliver strong suppressive signal selectively silencing these B cells and the subsequent allergic response.

Methods: protein engineering, intracellular signaling, FACS, animal models, ELISpot, ELISA, histology.

Results: Protein engineered chimeric molecules have been constructed, which bind Der p 1 specific B-cells via their BCR and suppresses selectively the production of anti-Der p 1 antibodies via the inhibitory B-cell receptors. The synthetic peptide Der p1 p52-71 and an anti-CR1 monoclonal antibody were used for the construction of human Der p1 chimera, and an anti-FcγRIIb antibody for the mouse chimera. The functional effects of engineered antibodies were analyzed in vitro. Studying intracellular signaling events we found that binding of the human chimeric molecule to tonsillar B cells triggers the tyrosine phosphorylation of a protein of 30-32 kDa, which is most likely involved in the inhibitory process. The chimeric molecules reduce the number of IgE anti-Dpt antibody producing plasma cells from allergic donors’ PBMC’s. High level of apoptosis was observed within CD19-gated PBMCs from allergic patients incubated with Der p 1-peptide chimera. We demonstrate that administering the chimeric molecule to immunodeficient SCID mice transferred with PBMCs derived from allergic patients results in reduction of allergen-specific IgE antibodies in the sera. A chronic murine model of HDM allergy has been developed and analyzed.

Discussion: The present study explores a different approach for preventing pathological allergen-specific IgE antibody production. Our data show that the allergic immune response can be silenced by specific targeting the pathological B cells.
THE EFFECT OF HUMAN RHINOVIRUS ON T REGULATORY CELLS

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Introduction: Respiratory infections with human rhinoviruses (HRV) are strongly associated with the development and exacerbations of asthma and pose a severe health risk for allergic individuals. How HRV infections and chronic allergic diseases are linked, and which role HRV plays in the breaking of allergen-specific tolerance is unknown. T regulatory cells (Tregs) play an important role in the induction and maintenance of immune tolerance. Therefore, the aim of this study is to investigate the effects of HRV on Tregs.

Methods: Healthy and asthmatic individuals were experimentally infected with HRV16 in vivo. Peripheral blood mononuclear cells (PBMCs) were obtained before infection and three days after infection. Tregs were sorted from the PBMCs according to the flow cytometric profile CD4+CD3+CD25+CD127- and were analyzed with next generation sequencing. Additionally PBMCs from healthy and asthmatic individuals were infected with HRV16 or stimulated with IFNα in vitro for 24 hours after which Tregs were sorted. Then the Tregs were co-cultured with naïve T cells and stimulated with αCD28 and αCD3 to assess their suppressive capacity. Furthermore qPCRs were performed to assess differences between stimulation with IFNα and HRV.

Results: We have found that on baseline there are clear differences in Tregs from asthmatics compared to healthy individuals. Tregs from asthmatics show a more Th2 type profile with increased expression of IL13, IL4, IL5, PTGDR2 and reduced FOXP3. After infection with HRV16 a strong antiviral response is induced in both Tregs from healthy and asthmatic individuals. The strongest induced genes are interferon induced genes such as MX1, IFI44L and OAS3. Interestingly in asthmatic individuals there is an additional upregulation of inflammasome genes and other virus related genes. In healthy individuals NR4A1-2 and 3, molecules important for Treg functioning, are upregulated while these are downregulated in asthmatic individuals. Furthermore there is upregulation of the suppressor molecules SOCS3, CTLA-4, CD69 and ICOS in healthy, while these are downregulated in asthmatics. With qPCRs we could confirm these findings in vitro and we could show that IFNα is an important driver of the antiviral response, but that IFNα alone is not enough to induce all genes. Lastly with Treg suppression assays we could show that upon RV stimulation Tregs loose some of their suppressive capacity.

Conclusions: Tregs from healthy and asthmatic individuals both show an anti-viral response after HRV infection. However there are clear differences in response between Tregs from healthy and asthmatic individuals in vivo. These differences in response might affect Treg functions, level of inflammation, chronicity and viral clearance. In vitro it was shown that after exposure to HRV16 Tregs lose some of their suppressive capacity. Together this data suggest that Treg functions might be altered or impaired during HRV infections and that this could play an important role in the association between HRV and the development of asthma and asthma exacerbations.
NEW AGE VACCINATION USING ASC SPECKS AS A CARRIER/ADJUVANT

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Introduction: Pathogen associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) are sensed by nucleotide binding oligomerization domain-like receptor (NLR) family of proteins in the cytosol. Certain NLRs like NLRP3 and AIM2 induce formation of inflammasome complexes. Apoptosis-associated speck-like protein containing CARD (ASC) is a 22 kDa adapter protein and has critical functions in inflammatory and pyroptotic signaling pathways by forming a bridge between pro-caspase-1 via CARD-CARD interactions and NLR's via pyrin-pyrin interactions and by activating caspase-1.

Aim: The aim of the purposed project is purifying ASC specks with antigen epitopes and achieving vaccination therefore developing ASC specks as a novel carrier and/or adjuvant modality. This project is secured by Turkish, Japanese, American (patent #6026645) and European Patenting Offices. In unstimulated cells, ASC is soluble in the cytosol; however, upon stimulation it forms globular speck structures in close proximity to the nucleus. The present invention aims to provide delivery of antigen-attached ASC specks to antigen presenting cells (APCs), enhancing the antigen presentation capacity of APCs to T cells, increasing the size of antigens to facilitate their engulfment by APCs, and increasing the shelf-life of antigens.

Method and Findings: In our preliminary studies, we managed to purify antigen-bound ASC specks with the model antigen ovalbumin (OVA) and also with mCherry protein as a tracker for imaging systems. I documented that purified ASC specks fed to macrophages via phagocytosis. Also, we showed that stimulation of macrophages with purified ASC specks significantly increases IL-1β and TNF-α secretion in cell. Afterwards, stimulation of immunity was checked by mice in vivo studies to demonstrate antigen delivery ability and adjuvant effect of ASC specks. Hemagglutinin is one of the most relevant antigens on influenza. Therefore, ASC specks conjugated with hemagglutinin H5 will be prototype for vaccine technology. I created most antigenic part of H5N1 (truncated H5) bound with ASC protein expressing plasmid and I transfected HEK293FT cells with the plasmid. Then, I successfully purified truncated H5 ASC specks and used them for immunization of mice. My fundamental ELISA results demonstrated that antigen-bound ASC specks inoculated mice had higher IgG levels rather than antigen with Alum (known adjuvant) immunized mice had. Additionally, I am using ASC specks (truncated-OVA bound) for tumor test as a novel targeted therapy. OVA expressing cells were inoculated subcutaneously in C57BL/6 mice. I demonstrated that intra peritoneal injected tOVA-ASC specks go to EG7-OVA thymoma and cause decrease in tumor volume.

Conclusion: We claim that our patented ASC speck delivery system can be useful in development of new age vaccines.
INVARIANT NATURAL KILLER T (iNKT) CELLS ARE NOT ESSENTIAL FOR MURINE NEUTROPHILIC ASTHMA PATHOGENESIS

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Asthma is a common chronic lung disease that results in inflammation and narrowing of the airways. Asthma is a heterogeneous disease and two main endotypes are distinguished according to the cells recruited to the airways. Eosinophilic asthma is a type 2-like immune response, characterized by the recruitment of eosinophils to the airways and the production of cytokines like IL-4, IL-5, and IL-13. In contrast, neutrophilic asthma is a type 1-like immune response, characterized by the recruitment of neutrophils and the cytokines IFNγ and IL-17A. The majority of asthma patients suffer from eosinophilic asthma and respond well to current treatments. However, the 5-10% of patients with neutrophilic asthma respond poorly. Invariant Natural Killer T (iNKT) cells have been described to enhance airway inflammation in an eosinophilic animal model of asthma. However, their role in neutrophilic asthma has not been clarified so far. We established mouse models to compare neutrophilic and eosinophilic asthma side-by-side and to study the role of iNKT cells in two different asthma endotypes. Using 19 parameter-flow cytometry, we analyzed cell frequencies, transcription factor expressions, and cytokine secretions of cell populations that are recruited to the inflamed lung and its draining lymph nodes. In eosinophilic asthma, severe eosinophilia, interstitial macrophage recruitment, and the production of cytokines, like IL-5 and IL-13 were detected. In contrast, in neutrophilic asthma, we observed a significant increase in neutrophil and exudate macrophage cell numbers and the production of IFNγ and IL-17A cytokines in the inflamed lung. Additionally, iNKT cell subset distribution was dominated by NKT1 cells in neutrophilic asthma, while in eosinophilic asthma, surprisingly, NKT17 cells were more abundant in the inflamed lung. Comparing WT and iNKT cell-deficient (Ja18-/-) mice, we found that iNKT cells do not affect the disease severity of neutrophilic asthma directly. Instead, in the inflamed lung, deficiency in iNKT cells reduced bronchus-associated lymphoid tissue (iBALT) formation. Currently, it is not clear why iNKT cells are heavily involved in eosinophilic but not in neutrophilic asthma pathogenesis. Hence, a better understanding of neutrophilic asthma is required to be able to develop novel therapies against this treatment-resistant type of asthma.
Molecular Mechanism for M1 Bias of ABCG1-Deficient Macrophages

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Macrophages that are major players of tumor immunity, are divided into two subgroups as M1 (pro-inflammatory) and M2 (anti-inflammatory) macrophages. In general, M1 macrophages are potent tumor-fighting cells, whereas M2 macrophages display protumoral functions. ATP-binding Cassette Transporter G1 (ABCG1) promotes cholesterol efflux from cells and regulates intracellular cholesterol homeostasis. Recently, we have shown that the absence of ABCG1 inhibits tumour growth in mice by modulating macrophage function within the tumour. In the absence of ABCG1, macrophages shift from an M2 phenotype to an M1 phenotype within the tumour and kill tumour cells directly. The molecular mechanism through which ABCG1-deficiency shifts macrophages to a tumor fighting M1 phenotype is not known. To address the molecular mechanism, we analysed cell signalling in bone marrow-derived macrophages from Abcg1−/− mice. These macrophages displayed reduced levels of Akt activation after stimulation with LPS/IFNγ or IL4. In addition, while Abcg1−/− macrophages stimulated with LPS/IFNγ produced more TNF compared to WT macrophages, after treatment with an Akt activator the TNF-production of Abcg1−/− and WT macrophages was comparable. These data suggest that the M1 bias of Abcg1−/− macrophages is mediated through the Akt signalling pathway. These findings not only deepen our mechanistic understanding of the M1/M2 switch in macrophages, but have the potential to open up new immunotherapeutic approaches for the treatment of cancer.
HUMAN UMBILICAL CORD TISSUE MESENCHYMAL STEM CELL (UCX®) EXOSOMES AS MODULATORS OF INNATE IMMUNITY

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Introduction: Mesenchymal stem cells (MSCs) are known to mediate immune responses apart from their well-known regenerative capacities. They have been shown to act as immunosuppressive agent in graft-versus-host disease and autoimmune conditions. Accumulating findings suggest that MSCs exert their paracrine effects through extracellular vesicles known as exosomes. Our previous data also revealed that exosomes isolated from 3 dimensional (3D) cell cultures are strikingly different in terms of protein content compared to exosomes from 2D cultures.

Aim: This study aimed to investigate the differential immunosuppressive capacities of 2D and 3D culture-derived UCX® exosomes on macrophages in terms of TLR activation and M1/M2 polarization.

Method: UCX® exosomes were isolated with ExoSpin size-exclusion columns. For TLR experiments, bone marrow derived macrophages (BMDMs) were stimulated with TLR 2, 4, 7 and 9 ligands Pam3CSK4 (1 μg/ml), LPS (1 μg/ml), R848 (1 μg/ml) or K23 CpG ODN (1 μM), respectively and treated with 20 μg/ml 2D or 3D UCX® exosomes for 24hrs. Cytokine concentrations were determined with ELISA and activation markers were analyzed with flow cytometry. For polarization studies, BMDMs were incubated with 20 μg/ml exosomes for 24hrs in the absence or presence of 100 ng/ml LPS for M1 induction or 10 ng/ml IL-4 and IL-13 for M2 induction. Nitric oxide (NO) levels in culture supernatants were detected with Griess Assay and M1/M2 polarization status via differential mRNA levels of target genes were determined with qRT-PCR. For the in vivo study, mice were intraperitoneally (IP) injected with 30 μg exosome/mouse and peritoneal exudate cells were collected 24hrs later. Myeloid-derived suppressor cell (MDSC) populations were analyzed with flow cytometry.

Findings: Both 2D- and 3D-exosomes significantly inhibited IL-6 and IL-12 secretions and to a lesser extent TNFα secretion induced by TLR activation. They also reduce surface markers such as MHC-II, CD80 and CD86. Overall, 3D-exosomes were more potent suppressors especially on TLRs 4 and 9. In the polarization assays, exosomes upregulated the M2 marker arginase 1 (Arg1) but inhibited the upregulation of another M2 marker Chil3 while also increasing the expression of M1 marker nitric oxide synthase 2 (Nos2) and NO secretion. This phenotype of high arginase and high NO production is similar to MDSCs. Moreover, when injected IP to mice, exosomes promoted local MDSC expansion. Although both types of exosomes have this capacity, 2D exosomes seem to be more potent inducers of MDSCs in vivo.

Conclusion: These results reveal that UCX exosomes were effective TLR antagonists. Furthermore, they shifted macrophages to an MDSC-like phenotype rather than an inducing an M1/M2 shift. Since MDSCs utilize arginase and NO to inhibit T-cell functions, further studies to reveal the potential of UCX® exosomes on T-cell inhibition is underway. Moreover, our data pointed out that 2D- or 3D-culture conditions result in exosomes with different therapeutic capacities. Overall, UCX® exosomes are novel suppressors of innate and perhaps adaptive immunity, therefore they might be valuable cell-free therapeutic agents for inflammatory and/or autoimmune disorders.

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AN IMMUNOSUPPRESSIVE OLIGODEOXYNUCLEOTIDE CONTAINING TELOMERIC TTAGGG MOTIFS ACT THROUGH PI3K/MTOR PATHWAY TO MODULATE IMMUNE CELL METABOLISM

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Introduction: A151 is a synthetic oligodeoxynucleotide (ODN) expressing telomeric TTAGGG motifs. We and others demonstrated that A151 ODN act as a TLR antagonist, notably by inhibiting STAT3 and AIM2- specific inflammasome activation. Mammalian target of rapamycin (mTOR) is the central protein integrating different signaling pathways by sensing internal and environmental factors such as cellular stress and nutrient levels, respectively. mTOR pathway activation has been shown to regulate cell growth, proliferation, protein synthesis and autophagy. Furthermore, it is the key protein modulating effector cell functions of various immune cells.

Purpose: Molecular mechanism of action of A151 ODN is still elusive, although its immunosuppressive effects were characterized in different immune cells. This study aimed to reveal how A151 ODN modulates PI3K/mTOR pathway.

Methods: Mouse splenocytes were induced with a CpG ODN (1 µM) in the absence or presence of A151 ODN (3 µM) for 8 hours, and gene expressions were assessed by RT-qPCR. Bone marrow-derived macrophages (BMDMs) and peritoneal exudate cells (PECs) were pre-treated with A151 ODN for 4 hours, then stimulated with LPS (100 ng/ml) to activate mTOR pathway. In parallel, rapamycin (100 nM) was used as positive control to inhibit mTOR phosphorylation simultaneously with LPS. mTOR phosphorylation levels were determined after 1-hour incubation with LPS via flow cytometry and immunoblotting. Finally, extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) were examined in A151 ODN treated BMDMs by Seahorse Analyzer to determine alterations in energy metabolism.

Results: RT-qPCR revealed that A151 down-regulated i) Pdpk1, Pi3ckcd and Rheb which are upstream elements of mTOR pathway, ii) mTOR itself, iii) Rps6ka1 which is one of the downstream targets of mTOR, iv) Myc, Slc2a1 and Stat3 whose mTOR-related genes. A151 pre-treatment significantly reduced mTOR phosphorylation in both mouse macrophages and B cells, and it was either equal or better than rapamycin mediated inhibition of mTOR phosphorylation. Down-modulatory effect of A151 ODN on mTOR phosphorylation was also validated by immunoblotting. Lastly, ECAR and OCR-specific metabolic analyses suggested that A151 ODN significantly decreased both glycolysis and oxidative phosphorylation levels of BMDMs.

Discussion: A151 ODN modulated mTOR both at transcriptional and at post-translational levels and down-regulated several mTOR related pathway elements. Although decrease in mTOR and Slc2a1, a gene coding for Glut1 implied A151 ODN might diminish glycolysis in macrophages, metabolic analyses studies revealed that it also lessened oxygen consumption rate. Despite the fact that impairments in both glycolysis and oxidative phosphorylation are only defined in T cells as “anergy”, herein we propose that A151 might transform macrophages to a state which is analogous to a T-cell anergy state, thereby acting as a metabolic regulator rather than an immunosuppressor.

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A SYSTEMIC COMPARISON OF DIFFERENT CHIMERIC ANTIGEN RECEPTOR (CAR) DESIGNS FOR RETARGETING OF NK-92 CELLS AGAINST TUMOR ANTIGENS

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Introduction: Cancer immunotherapies focus on the power of the immune system to attack tumors. Recently, Chimeric Antigen Receptors expressing T cells (CAR-T cells) have received clinical approval for antigen-specific adoptive immunotherapy against CD19 in B cell malignancies. CAR vector designs have dramatically developed since their initial discovery and now include first generation CARs (CD3ζ-based CAR), second generation CARs with an additional costimulatory domains such as CD28 or CD137 and third generation CARs (CD3ζ with two costimulatory domains). CAR expression in NK cells is also clinically tested and carries the potential to translate into clinical application.

Purpose: To show the effects of using different signaling domains including CD3ζ, CD28 and CD137 in triggering CAR-mediated activity of NK-92 cells against CD19 positive cell lines such as B-cell lymphoma cell line -Raji and B-cell lymphoblast- Daudi.

Methods: CARs 63.z, 63.28.z and 63.137.z vectors (Oelsner, 2017) were provided by Winfried S. Wels, Georg-Speyer-Haus Institute Germany. Lentiviral particles were produced using HEK 293FT cells and NK-92 cells were transduced. EGFP-positive NK-92 cells were sorted by FACS. Degranulation of CAR-NK-92 cells upon exposure to CD19+ and CD19- target cells was measured by analysis of surface expression of lysosomal-associated membrane protein LAMP-1 (CD170a).

Results and Discussion: NK-92/63. 137.z cells were less effective than NK-92/63.z and NK-92/63.28.z in showing cytotoxic activity against CD19+ targets. This may in part be explained by differences in the threshold for CAR signaling. CAR 63.z utilizes the endogenous transmembrane domain of CD3ζ, which allows formation of disulfide linked CAR homodimers and heterodimers of CAR and endogenous CD3ζ.

CHARACTERIZATION OF FOOD ALLERGEN-SPECIFIC B CELLS BEFORE AND AFTER ORAL IMMUNOTHERAPY

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Background: The prevalence of food allergy is an increasing public health concern affecting millions of people worldwide. Besides their role in the production of allergen-inducing IgE antibodies, allergen specific B cells may play a role in the induction of allergen tolerance. The aim of this study was to examine the role of B cells in cow’s milk allergy.

Methods: Peripheral blood mononuclear cells (PBMC) from cow’s milk allergic patients, who followed the clinical Food allergen-specific immunotherapy (Food-AIT) were obtained before and 18-35 months after the Food-AIT. αS1-casein specific and non-specific B cells were identified and purified using dual-color staining with fluorescently labeled αS1-casein allergen by flow cytometry. The immortalization of αS1-casein specific B cells were performed through transduction with a retroviral vector containing GFP, BCL6 and Bcl-xL and expanded by culturing with CD40L and IL-21. Total and specific IgE, IgG and IgG subclass (IgG1, IgG2, IgG3, and IgG4) antibodies from culture supernatants of immortalized B cells were measured by ELISA. The single cell/ Ultra Low RNA next generation sequencing was performed for quantitative transcriptomics.

Results: After purification of αS1-casein specific B cells and non-specific B cells, we measured the Ag-specific Ig profile to confirm their specificity. Specific IgE, IgG1 and IgG4 production from culture supernatants of αS1-casein positive B cells was significantly elevated compared to αS1-casein negative cells, while total IgE, IgG1 and IgG4 levels were comparable. The in-depth analysis of gene expression showed significantly different between αS1-casein specific B cells and non-specific B cells. Interestingly, most of transcription factors that involved in the regulation of cell proliferation, including ZNF41, ZNF140, ZNF268, and STAT4 which is a part of the inflammatory response are downregulated in αS1-casein specific B cells after Food-AIT. Additionally, chemotactants CXCL1/CXCL2 gene, which control the neutrophil recruitment during tissue inflammation and inflammatory cytokine receptors, which bind to cytokines that promote inflammation including IL-12RB1 and IL18R1 are also downregulated.

Conclusions: This study is focused on the characterization of allergen-specific B cells in cow’s milk allergen. The in-depth analysis of these cells in patients with Food-AIT in terms of gene expression (using RNAseq) suggesting less cell proliferation, less neutrophil recruitment and less inflammatory responses in patients with cow’s milk allergy after Food-AIT. Keywords: allergen-specific B cells, alpha S 1 casein, cow’s milk, food allergy, RNA sequencing.
IMMUNE MODULATORY THERAPEUTIC APPLICATIONS OF PEDIOCOCUS PENTOSACEUS DERIVED MEMBRANE VESICLES

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Gram negative and gram positive bacteria constitutively secrete membrane vesicles that are enriched in TLR ligands. Previously, we characterized 5 different gram positive commensal bacteria derived MVs and compared their immunomodulatory activities with gram negative non-pathogenic E.coli derived outer membrane vesicles. Our results showed that, commensal but not E.coli derived derived MVs had immunoregulatory effects. Herein, we present our findings on the mechanism of immunosuppression mediated by Pediococcus pentosaceus derived MVs. In an immunization model with OVA model antigen, Pediococcus pentosaceus derived MVs supressed anti-OVA specific IgG2c and CTL responses. Analysis of MV effect on different cell types showed that MVs exerted an immunomodulatory response by generating M2 macrophages and myeloid derived suppressor cells (MDSCs) but not regulatory T cells. MVs’ anti-inflammatory effects were also tested in acute inflammation models established in mice. In a zymosan-induced peritonitis model, MVs ameliorated excessive inflammation by reducing neutrophil recruitment to peritoneal cavity and inhibiting macrophage loss caused by inflammation. In dextran sodium sulphate (DSS) induced acute colitis model, post-treatment with MVs (Day 0 and 3) prevented colon shortening and loss of crypt architecture. In an excisional wound healing model, intraperitoneal MV administration accelerated wound closure through recruitment of PD-L1 expressing myeloid cells to the wound site. Collectively, these results indicate that Pediococcus pentosaceus derived membrane vesicles activate myeloid-derived suppressor/regulatory cell types and can be used as potent anti-inflammatory agents for the treatment of inflammatory or autoimmune diseases. This project was partially supported by a TUBITAK grant (113S305).
RAP1/RIAM MODULE CONTROLS QUANTITATIVE AND QUALITATIVE PROPERTIES OF EFFECTOR-MEMORY AND REGULATORY T CELLS

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Introduction: Integrins play a central role in many aspects of T cell biology, such as cell migration, adhesion, and the immune response. Owing to their critical role in T cells, activation of integrins is finely tuned through a process called “inside-out signaling”. Rap1 is a small GTPase with a crucial role in canonical signaling events in hematopoietic cells. Integrin activation represents one of the best studied cellular processes in which Rap1 is implicated with its downstream effectors Rap1-interacting adaptor molecule (RIAM) and RapL. RIAM regulates integrin activation by altering the conformation of leukocyte function-associated antigen-1 (LFA-1) beta chain. While the role of Rap1/RIAM-mediated integrin activation in cell adhesion and lymphocyte trafficking has been extensively studied, the value of Rap1/RIAM module to effector and regulatory T cell compartments remains unclear.

Aim: In this study, we aimed to investigate the contribution of Rap1 signaling axis and its downstream effectors in T helper cell differentiation and regulatory properties of the immune system.

Method: We employed mice expressing a constitutively active form of Rap1-GTP in T cells with or without conditional knock-out of RIAM or LFA-1. Single cells suspensions were prepared from thymus, spleen, pooled peripheral LNs (brachial, inguinal, axillary and superficial cervical) and/or mesenteric LNs. Using these isolated immune cells from experimental mice, we performed several functional in vitro and in vivo T cell activation, differentiation and suppression assays. Flow cytometric characterization of the key T cell subsets in lymphoid organs were also carried out under homeostatic and immunization conditions by means of CD8a, CD4, TCRb, CD3, CD62L, PD-1, ICOS, CD44, LFA-1, CD25, CD103, CD80, CD86, Foxp3, CD73, CD39, CTLA-4, CXCR5, GITR expressions.

Findings: We found that forced expression of active Rap1 promoted effector-memory (Tem) and regulatory T cell (Treg) differentiation in vivo, while RIAM loss resulted in diminished Tem and Treg cell proportions under steady-state conditions. Further subset analyses revealed that Th17 and T follicular helper (Tfh) cells within Tem compartment were enhanced in secondary lymphoid organs of Rap1-GTP mice including Peyer's Patches. Mechanistically, the reduction of Tem and Treg abundance in RIAM-deficient mice was attributed to the abrogated immune-synapse formation and TCR signaling due to the impaired activation of LFA-1. Moreover, the suppressive capacity and effector differentiation of Treg cells were found to be highly dependent on Rap1/RIAM module.

Conclusion: Taken together, our study identifies a novel implication of Rap1/RIAM signaling axis in controlling Tem and Treg cell pool by promoting their maintenance, development, and functionality.
EFFECTS OF CANCER ASSOCIATED FIBROBLASTS ON MONOCYTES AND MACROPHAGES IN BREAST CANCER

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Introduction: Fibroblasts are one of the most abundant cell types found in the tumor stroma. Fibroblasts turn into cancer associated fibroblasts (CAFs) in the tumor microenvironment. Monocytes originating from blood differentiate into either M1 or M2 subtype macrophages, depending on the environmental stimuli. Macrophages have roles in almost all stages of tumor progression. Tumor associated macrophages usually resemble M2 macrophages. The identification of molecules driving macrophage plasticity in the cancer microenvironment could provide a basis for macrophage focused diagnostic and therapeutic strategies.

Purpose: The aim of the current study is to determine the role of CAFs on monocyte recruitment and macrophage polarization in breast cancer.

Method: Fibroblast cells were isolated using Collagenase I (1 mg/mL) and Hyaluronidase (125 U / mL) enzymes followed by differential sedimentation and plating. Characterizations of fibroblasts were performed by immunocytochemistry stainings with vimentin, pan-cytokeratin and α-SMA. Conditioned mediums (CMs) were obtained from CAFs and NFs. PBMCs were isolated from healthy volunteers’ peripheral blood using density gradient separation with Histopaque-1077. Separation of CD14+ monocytes and CD4+ T-cells from PBMCs was accomplished by using magnetic bead based selection protocols. Cells were stained with antibodies against CD206, CD163; and analyzed with flow cytometry. Functional effects of CAF and NF educated monocytes on CD4+ T lymphocytes were analyzed with flow cytometry utilizing carboxyfluorescein succinimidyl ester (CFSE) labelled CD4+ T-cells that were activated by CD3 / CD28 magnetic beads. The effects of CAFs and NFs on monocyte recruitment were investigated by in vitro migration assays with Transwell chambers. E-cadherin and vimentin protein expressions were examined by Western blot. MDA-MB-231 breast cancer cell invasion was evaluated by using Transwell inserts in vitro.

Results: Our results demonstrated that CAFs express α-SMA, while normal fibroblasts (NFs) do not. CAFs were shown to effectively recruit monocytes. This recruitment may be mediated by MCP-1 or SDF-1 cytokines, since inhibition
SMALL CELL LUNG CANCER STEM CELLS SHOW ADAPTIVE RESISTANCE THROUGH EFFECTIVELY INDUCING T CELL ACTIVATION AND MODULATING THE EXPRESSION OF CO-INHIBITORY RECEPTORS

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Introduction and Aim: Tumors are capable of reshaping its microenvironment to escape from immune destruction. For this purpose, tumor cells initiate an immune regulatory program that is typically used by the normal cells to mitigate immunopathologies. Small cell lung cancer (SCLC) is associated with aggressive tumor growth together with a tendency to early dissemination and distant metastasis. Optimal drug responses are observed upon conventional platinum-based chemotherapies; yet, resistance develops after a short period of therapy. These facets of SCLC bring forward the cancer stem cell concept. Recent studies described the candidate stem cell populations of SCLC. Although mesenchymal stem cells are stated to be immune-tolerogenic, less is known how SCLC stem cells interact with immune cells. Thus, the aim of the study is to define the relationship between T cells and SCLC cells including its stem cell population and reveal their immunomodulatory capacities.

Methods: SCLC cell lines (NCI-H82, NCI-H69, SCLC-21) which are cultured as suspensions, adherent mesenchymal-like subsets isolated from NCI-H82 and NCI-H69, and CD44+CD90+ subpopulation from adherent fraction of NCI-H69 were used. Cancer stem cell characteristics were confirmed by chondrocyte, osteocyte and adipocyte differentiation and transcriptomic analysis by RNA-seq. PBMCs obtained from healthy volunteers were co-cultured with these subpopulations of SCLC cells in the presence of α-CD3 and expression of activation markers (CD25, CD107a, 4-1BB and CD69), proliferation rate, cytokine production (IL-2 and IFN-γ) and upregulation of co-inhibitory receptors (PD-1, CTLA-4, TIM-3 and LAG-3) were determined on CD8+ T cells. PD-L1 and PD-L2 levels were measured on SCLC cells upon IFN-γ exposure or PBMC co-culture with or without IFN-γ blockade. The impact of PD-L1 expression on CD8+ T cell proliferation and T cell killing of SCLC cells were determined. Functional capacity of TIM-3+LAG-3+ and TIM-3+LAG-3-CD8+T cells upon SCLC co-culture were assessed in vitro.

Findings: SCLC cells allowed or even supported T cell responses. This effect was more prominent with the stem cell subpopulations of SCLC. Furthermore, SCLC stem cells displayed adaptive resistance by inducing PD-L1 and PD-L2 upon exposure to IFN-γ and activated PBMCs. Uprogulation of co-inhibitory receptors PD-1, TIM-3 and LAG-3 on CD8+ and CD4+ indicated that in the presence of SCLC cells, T cells became available for receiving inhibitory signals such as PD-L1 expressed on SCLC stem cells. The presence of PD-L1 on these cells was correlated with decreased proliferation of CTLs and resistance against T-cell killing.

Conclusion: The stem cell subpopulation in SCLC possesses significant immune regulatory capacities. These cells display adaptive resistance, express inhibitory ligands and at the same time induce the expression of co-inhibitory receptors on T cells. Therefore, targeting the immunomodulatory capacity of cancer stem cells might be considered for the immunotherapy approaches in SCLC.

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IgE PRODUCTION IN CHRONIC GRANULOMATOUS DISEASE RELATED TO FUNGAL ANTIGENIC STIMULATION

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Introduction: Chronic granulomatous disease (CGD) is an inherited disorder of phagocytes in which affected patients suffer from recurrent bacterial and fungal infections. It is defined as the inability to create a normal respiratory burst in response to microbial stimulus. We observed that high serum IgE formation can be detected in some of the CGD patients during diagnosis or follow up. However, the cause of higher IgE production is not well known.

Aim: In this study, we aimed to investigate the effect of in vitro antigenic stimulation of Aspergillus and Candida on IgE class-switch recombination from naive B cells in CGD patients.

Method: CGD patients (n=10) and healthy controls (n=7) were included to the study. Serum total IgE, specific IgE and skin prick test were conducted. Naive B cells were isolated and incubated for 7 days with the following conditions: (i) Unstimulated media (cRPMI+ naive B cells), (ii) Naive B cells+CD40L+HA-peptid+IL-4+IL-21 (IgE media), (iii) Naive B cells+CD40L+HA-peptid+IL-4+IL-21+Heat-Aspergillus, (iv) Naive B cells+CD40L+HA-peptid+IL-4+IL-21+Heat-Candida. After 7 days, culture supernatants were collected and IgE production was measured by ELISA.

Findings: When compared to healthy controls, in vitro IgE production was statistically higher both in conditions (ii) and (iii) (p=0.035 and p=0.032, respectively) in CGD patients. When CGD patients were evaluated separately, both Aspergillus (condition iii) and Candida (condition iv) stimulations exhibited increased IgE production compared to IgE media (p=0.0002 and p=0.038, respectively). In addition, in CGD patients, IgE production with Aspergillus stimulation was significantly higher than Candida stimulation (p=0.0003). In healthy controls, in vitro IgE production in condition (iii) was also higher compared to condition (ii) (p=0.0006).

Conclusion: In CGD patients, chronic exposure to Aspergillus could be associated with high IgE production. This work was supported by Marmara University research project BAPKO No: SAG-A-110915-0410.
REJECTION IN AN IMMUNE PRIVILEGED TISSUE: CHARACTERISTICS OF IMMUNE CHECKPOINTS AND LYMPHOCYTE SUBSETS IN CORNEAL REJECTION

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Introduction: Corneal transplantation can be performed without the need of immunosuppressive treatments and HLA matching, due to the ocular immune privilege. Despite this special immunological environment, corneal rejection can occur in around 10% of the cases.

Purpose: The aim of this study was to define the characteristics of immune checkpoints and lymphocyte subsets in the tissue rejection within the setting of immune privilege, in the example of corneal rejection.

Method: Corneal tissues and aqueous humor samples were taken from nine chronic corneal rejection patients. Blood samples obtained from 12 healthy blood donors, corneal tissues from three healthy donor rims and aqueous humor samples from seven cataract surgery patients were included in this study. Corneal infiltrating lymphocytes (CIL) were demonstrated by immunohistochemistry and further characterized by flow cytometry. The aqueous humor levels of soluble PD-1, soluble PD-L1 and soluble CTLA-4 were determined by ELISA.

Findings: Histopathological evaluation of the rejected corneas demonstrated lymphocyte infiltration. In flow cytometry, B cells constituted 36.0% of the CIL, followed by NK T (23.3%), CD8+ T (21.4%) and NK (19.1%) cells. CD4+ T cells were low in number (1.3%). Composition of aqueous humor lymphocytes was also similar (26.4% CD8 T, 25.0% NK, 18.6% B, %13.1% NK and 5.6% CD4+ T cells). PD-1 expression was high in all CIL lymphocyte subsets compared to aqueous lymphocytes (p<0.05). The level of PD-1 expression was lower in innate lymphocytes (29.2% in NK T and 22.6% in NK cells) compared to adaptive lymphocytes (61.5% in CD8+ T and 60.2 in B cells). CD16/CD56/dim cytokine secreting NK subset was increased in corneal rejection (p=0.001). CD16/bright/CD56/dim cytotoxic NK subset was reduced in ocular tissues (p=0.001). All NK subsets from ocular tissues had increased expression of PD-1 (p<0.05), but PD-1 expression was slightly lower in the cytokine secreting subset. Aqueous sPD-1, sPD-L1 and sCTLA-4 levels were significantly higher compared to healthy plasma samples (p<0.01). There was no significant difference for sPD-L1 (3.2 ng/ml vs. 3.2 ng/ml) and sCTLA-4 (36.6 ng/L vs. 39.3 ng/L) levels between the rejection and cataract groups, but sPD-1 (102.9 µmol/L vs. 77.0 µmol/L) was significantly higher in the rejection group (p=0.037).

Conclusion: To the best of our knowledge, this is the first study to report a detailed analysis of lymphocyte subsets and immune checkpoints during immune rejection at an immune privileged site in humans. It was interesting to note that CD4+ T cells were underrepresented among CIL. NK cells, NKT cells and B cells predominated in human corneal rejection. The coexistence of B cells and NK cells has the potential to end with antigen dependent cellular cytotoxicity.
Cytokine secreting NK subset is further increased within the NK population, which might have taken over the role of CD4+ T cells here. There was no defect in studied immune checkpoints. These results indicate an innate signature in human corneal rejection.
ONCOLYTIC IMMUNOVIROThERAPY TO TREAT COLORECTAL CARCINOMA

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Introduction: Oncolytic immunovirotherapy is a novel form of therapy for the treatment of cancer. Potential of this method has been proven in several clinical trials in the last two decades. Oncolytic viruses selectively replicate in cancer cells, spread within tumor tissue and lead to tumor destruction. This virus-mediated cell killing results in release of tumor associated antigens (TAAs), danger associated molecular patterns (DAMPs) and pathogen associated molecular patterns (PAMPs) to the tumor microenvironment (TME) which in turn lead strong innate and adaptive anti-tumor immune responses. Oncolytic potential of many different DNA and RNA viruses have been investigated. Coxsackievirus B3 (CVB3), a single-stranded RNA virus, has been described as a novel oncolytic virus for different cancer types. The immunostimulatory effect of CVB3 was shown by detection tumor infiltrating lymphocytes two days after intratumoral virus administration. However, mild side effects were observed in treated animals. Recently, we described another CVB3 strain (CVB3-PD) which have different receptor tropism. Cell receptor-virus interaction is the major factor of tumor selectivity for oncolytic viruses. While majority CVB3 strains use coxsackievirus and adenovirus receptor to infect the cells, CVB3-PD use tumor cell up-regulated heparan sulfate (HS).

Aim: It was aimed to investigate oncolytic potential, safety profile and immunostimulatory effect of HS-binding CVB3-PD using xenograft and syngeneic colorectal cancer mouse models.

Method: To evaluate oncolytic efficiency of CVB3 strains in vivo, a xenograft BALB/c nude mouse tumor model was established with the DLD1 human colorectal carcinoma cell line. Tumor cells were inoculated bilaterally into the flanks of the animals, and only one of the two tumors was injected with single dose of CVB3 PD. Next, we analysed oncolytic activity of CVB3-PD in intact immune system, in syngeneic mouse model. Murine colorectal cancer Colon-26 cells were inoculated subcutaneously into the right flank of the immunocompetent BAL/c mice. Tumor size was measured daily. In the end of the experiment, animals were sacrificed and tumors were harvested. Immune cells were isolated and analyzed by FACS.

Findings: Intratumoral injection of PD into the colorectal tumors in nude mice each resulted in strong tumor regression both in injected and in a non-injected, contralateral tumor. Viral RNA was detected in tumor infiltrating immune cells ten days post infection. No systemic infection was observed in PD infected animals. Next, we analysed oncolytic activity of CVB3-PD in intact immune system, in syngeneic mouse model. Strong tumor regression was observed in all treated animals and infiltrating immune cells were detected 17 days post virus infection. No side effects was observed in any treated animals.

Conclusion: In this study, CVB3-PD was demonstrated as a safe and potent oncolytic virus. Specific receptor tropism and non-toxic tumor selective feature of the virus even in athymic mice, stimulation of anti-tumor immune response in immunocompetent mice underline the potential of PD as an oncolytic agent.
POSTER ABSTRACTS
PLASMODIUM AND NEOSPORA OTU PROTEINS BLOCK NF-KB, INTERFERON AND PYROPTOSIS PATHWAYS

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OTU deubiquitinases are key proteins to antagonize any cellular defense pathways post entry of intracellular obligatory parasites into the host cell. We previously studied CCHFV OTU protein, determined its potent inhibitors and inhibition pocket. Intriguingly, three of malaria causing parasitic Plasmodium species and neospora caninum pose previously uncharacterized proteins similar to viral OTU protein. These viral OTU-like proteins have highly conserved aminoacid residues in the catalytic and inhibition pockets similar to viral OTU protein. However, structure, inhibitors or function of Plasmodium and Neospora OTU-like proteins have not been previously determined. To this end, we have performed modeling, orphan drug analysis, deubiquitinase assays, western blot analysis, gene expression analysis and determined potent inhibitors of OTU-like proteins of P. falciparum, P. vivax, P. yoelii and N. caninum species. We have recombinantly expressed C-His-tagged OTU-like proteins in E. coli and purified them by affinity chromatography. OTU activity and potent inhibitors were determined using fluorescent UB-AMC deconjugation assay. PCDNA3.1 OTU vectors were transfected into HEK293 mammalian cells to determine intracellular deubiquitinase activity of plasmodium and neospora OTU proteins. The effects of plasmodium and neospora OTU proteins on mono-ubiquitination and mono-poly-ubiquitination profiles in HEK mammalian cells were determined by western blot. Plasmodium and neospora OTU proteins in the human cells increased expression of dsDNA and dsRNA marker genes, significantly reduced the CASP1 gene expression involved in the pyroptosis pathway, decreased expression of majority of the genes of involved in cellular immunity, and suppressed interferon stimulating genes APOBEC3G and MX1 at very high rates. This has also shown that plasmodium and neospora OTU proteins primarily block intracellular immune response by controlling the expression of genes in the NF-kB and interferon pathway. These findings suggest that targeting plasmodium and neospora OTU proteins is a plausible strategy to develop new anti-malarial and anti-neosporosis therapies.

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IMPLICATION OF SOLUBLE HLA-G AND HLA-G +3142G/C POLYMORPHISM IN BREAST CANCER PATIENTS RECEIVING ADJUVANT THERAPY AT ORCI, TANZANIA

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Introduction: Sufficient evidence exists on the protective role of immune system against cancer. HLA-G is an immunosuppressive molecule existing in a soluble (sHLA-G) and membrane-bound form. It is thought to be expressed more by tumor cells and hence contributing to immune evasion. The HLA-G +3142G/C polymorphism (rs1063320) post-transcriptionally affects the expression of HLA-G. This molecule and SNP have been implicated in breast cancer (BC) and others, but with limited studies in African population. Purpose: The aim of the study was to investigate how sHLA-G and rs1063320 SNP are related to breast cancer (BC) in Tanzanian population.

Methods: The study consisted of 75 BC patients and 84 normal controls. Most BC patients were under medication and 81.3% had undergone mastectomy. Plasma sHLA-G was quantified by ELISA. The genotyping was done by LightSNiP typing assay using quantitative Real-Time PCR. The data for rs1063320 genotyped from African related populations (Luhya, Maasai, Yoruba, African ancestry-people in Southwest USA, data generalizing all African populations) were downloaded from phase-3 1000Genomes and HapMap databases.

Results: The sHLA-G level was significantly lower in patients and mastectomized patients as compared to controls (p<0.01) and non-mastectomized patients (p=0.018) respectively. sHLA-G was not relevant to metastatic and receptors expression (ER, PR and HER2) status. The frequencies distribution of rs1063320 genotypes and alleles were relatively similar between patients and controls. There was no significant influence of rs1063320 genotype on sHLA-G level. The Kaplan-Meier analysis revealed no significant difference between patients carrying at least one risk allele versus no risk allele(GG) in their likelihood to have metastatic free survival (Log rank, p=0.6508). With the exception to Luhya population from Kenya (95% CI, p>0.05), the genotypic and allelic distribution pattern of this locus in this study population was significantly different from the rest four African related populations (95% CI, p<0.01).

Discussion: Some evidence shows that medical intervention particularly BC surgery followed by adjuvant therapy significantly lowers sHLA-G level among BC patients (Sayed D. et al 2010). The unexpected low level of sHLA-G in BC patients may plausibly be attributed to such medical intervention, as 81.3% of BC patients had undergone mastectomy. Similarly, non-mastectomized patients had higher sHLA-G than mastectomized patients. Such influence reflects the possible utility of sHLA-G as a prognostic marker. Multiple genetic variants in 3'UTR and promoter regions affect the expression of HLA-G. This may explain the lack of influence of rs1063320 genotype on sHLA-G level observed in this study. Similar to the differences in allelic and genotypic frequencies distribution observed between our study population and other African related populations, differences in Linkage-Disequilibrium patterns among 3'UTR genetic variants were observed among geographical groups world-wide (Sabbagh A, 2014). This might be the basis of conflicting findings over association between rs1063320 and BC and other cancers.

Conclusion: This study suggests that, while changes in sHLA-G levels in response to medical interventions such as mastectomy may be translated into its potential use as a prognostic marker for BC, the rs1063320 may not solely affect the sHLA-G level and reliably serve as a genetic risk factor for BC.
LONG NONCODING RNAs ASSOCIATED WITH FATALITY IN CRIMEAN CONGO HEMORRHAGIC FEVER DISEASE

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Crimean-Congo hemorrhagic fever (CCHF) is a widespread tickborne disease that can progress from mild fever and nonspecific signs to a severe and often fatal hemorrhagic disease in infected humans. Long noncoding RNAs (lncRNAs) form a large category of the RNA world, have a direct or indirect influence in gene regulation. Several lncRNAs whose expression is altered by infection have been described as a new perspective of viral diseases. Therefore, we investigated lncRNA expression profiles in fatalities due to CCHF using microarray analysis at first time in the world. Blood samples were collected into the RNA blood tube. The lncRNA isolation was performed according to the PAXgene Blood RNA Kit. The RNA integrity numbers were detected at Bioanalyzer. LncRNA gene expression was determined using SurePrint G3 Human gene expression v3. Gene Spring program was used for bioinformatics analysis. One Hundred Eleven lncRNAs were statistical significant in fatalities compared to the controls (11 were upregulated, 100 were downregulated). The highest downregulated lncRNAs were lnc-AP001496.1-10 (FC: -606.71), lnc-LSR-1 (FC: -446.30), LINC00644 (FC: -397.09) while the highest upregulated lncRNAs were ECRP (FC: 51.42), LOC100133669 (FC: 24.71) and lnc-TRIM16-1 (FC: 20.01). Significant lncRNAs can behave as inducers or repressors of the antiviral response and play effective role in disease progression. They also can be thought therapeutic agent to prevent the death of patients with CCHF. Note: This work was supported by The Scientific and Technological Research Council of Turkey (TUBITAK, Project code-no: 1001-117S146), Ankara, Turkey.
ANTITUMOR ACTIVITY OF GASTROPODAN HEMOCYANINS IN C-26 MURINE MODEL OF COLON CARCINOMA

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Introduction: A number of natural compounds are designed to repair, stimulate, or enhance the immune system response. Among them are the hemocyanins (Hcs) - extracellular copper proteins isolated from different arthropod and mollusc species. The hemocyanins (Hcs) are oligomeric copper-containing glycoproteins that function as oxygen carriers in the hemolymph of these animals. Molluscan Hcs have been studied intensively for many years as very promising class of anti-cancer therapeutics, generating strong humoral and cellular immune response. A possible mechanism for their antitumor effect is the presence of cross-reactive epitopes between the carbohydrate content of the hemocyanin molecule and tumor-associated carbohydrate antigens, which are characteristic for different types of cancer. Murine colon carcinoma cell line C-26 is well established model for tumor introduction in mice.

Purpose: The aim of the present work was to develop an experimental murine model of colon carcinoma and to investigate the anti-tumor activity of two new hemocyanins.

Method: The Hcs were isolated from marine snail Rapana thomasiana (RtH) and the terrestrial snail Helix pomatia (HpH). Both Hcs were further purified by gel filtration chromatography and were passed through Detoxi-Gel column for endotoxin removing. Murine colon carcinoma cell line C-26 was used for animal administration and solid tumor establishment. Recently, we have shown that the RtH and HpH possess high immunogenicity and can be used as anti-cancer agents in C-26 murine model of colorectal carcinoma using two schemes of immunization. Tumor growth was monitored by measuring palpable solid tumors once weekly and the tumor volume was estimated with a microcaliper. Flow cytometry was performed for phenotyping of spleen and tumor suspensions and an apoptosis assay. The levels of cytokines and anti-C-26 antibodies were quantified by ELISA. ELISpot assay for counting specific anti-C-26 IgG antibody-secreting cells was also performed. Detection of tumor antigen cross-reactivity was performed by dotblot assay for SLeX antigen presence on C-26 lysate, RtH and HpH.

Results: The Hcs exhibited strong in vivo anti-cancer and anti-proliferative effects in the developed murine model of colon carcinoma. We observed a significant increase of the spleens in non-treated C-26-bearing mice compared to Hcs treated. The immunization with RtH and HpH prolonged the survival of treated animals, improve humoral anti-cancer response and moderate the manifestation of C-26 carcinoma symptoms as tumor growth, splenomegaly and lung metastasis appearance. It was observed a crossreactivity between Hcs and C-26 cells, further tumor-associated SLeX antigen presence on C-26 lysate, RtH and HpH was detected.

Discussion: Hemocyanins are used so far for therapy of superficial bladder cancer and murine melanoma models. Our findings demonstrate a potential anti-cancer effect of hemocyanins on a murine model of colon carcinoma suggesting their use for immunotherapy of different types of cancer.
IL-22 -REG3γ AXIS MODULATION IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS AND IMPLICATIONS FOR HUMANS MULTIPLE SCLEROSIS

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Introduction: IL-22 is a member of IL-10 cytokine family and is produced mainly by RORγt+ innate and adaptive lymphocytes, including ILC3, γδ, iNKT, Th17, and Th22 cells. Heterodimeric IL-22 receptor, however, is expressed primarily by non-hematopoietic cells. Reg3γ is an antimicrobial peptide produced by intestinal epithelial cells upon stimulation with IL-22. Both IL-22 and Reg3γ are critical for barrier immunity at the mucosal surfaces in the steady state and during infection.

Aim: Although IL-22 knock out mice were previously shown to develop experimental autoimmune encephalomyelitis (EAE), a murine model of multiple sclerosis (MS), how IL-22 or Reg3γ manipulation in adult mice would affect EAE course has not been studied. We aimed to investigate this question in murine MOG35-55 induced EAE model by overexpressing IL-22 or Reg3γ or neutralizing them.

Methods: In this study, we over-expressed IL-22 via hydrodynamic gene delivery or blocked it via neutralizing antibodies in C57BL/6 mice. Similarly, we overexpressed Reg3γ to assess the impact of IL-22-Reg3γ modulation on EAE course. In the MOG35-55 induced EAE model, EAE scores, infiltrating T helper subsets to the central nervous system (Th17, Th1, Treg) was quantified by intracellular cytokine staining. Demyelination in the spinal cord was assessed by Luxol Fast Blue staining. We also examined the expression patterns of Reg family genes and their potential receptors, Exostosin (Ext) family members, in the brain tissues obtained from naïve and EAE mice as well as cerebrospinal fluid (CSF)-derived cell lysate obtained from MS patients and healthy controls via real time qPCR.

Results: IL-22 overexpression significantly but marginally decreased EAE scores, demyelination and reduced infiltration of IFNγ+IL-17A+ Th17 cells into central nervous system (CNS). In contrast, neutralization of IL-22 exacerbated EAE scores. Surprisingly, overexpression of Reg3γ, an epithelial cell-derived antimicrobial peptide induced by IL-22, significantly exacerbated EAE scores, demyelination and infiltration of IFN-γ+IL-17A+ and IL-17A+GM-CSF+ Th17 cells to CNS. Finally, we show differential gene expression of Reg and exostosin family members in the brain of healthy and EAE mice as well as CSF of human MS patients and non-MS controls.

Discussion: Our results provide novel insight into the role of IL-22 and Reg3γ in the pathogenesis of CNS inflammation in a murine model of MS and potentially human MS.
CHARACTERIZATION OF TREG CELLS GENERATED IN THE PRESENCE OF GROUP 3 INNATE LYMPHOID CELLS EX VIVO

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Introduction and Aim: CD4+ Foxp3+ Treg cells are a heterogeneous population of suppressive helper T cell lineage which includes thymically and extrathymically derived subsets. Ex vivo generated polyclonal or antigen specific induced Treg (iTreg) cells are utilized for therapeutic purposes in autoimmune disease models. Group 3 innate lymphoid cells (ILC3s) are Rorγt+ innate cell population enriched at the mucosal surfaces. ILC3s also express MHCII and interact with CD4+ T cells, however, if and how ILC3s may regulate Treg cell functions has not yet been addressed. In our study, we characterized the molecular features of Treg cells generated from naive CD4+ T cells in the presence or absence of ILC3s ex vivo.

Methods: CD4+ Foxp3- T cells were sorted from Foxp3YFP reporter mice. ILC3s were purified from IL-23 receptor GFP reporter mice. Treg differentiation was performed with TGF-β, IL-2 and CD3/CD28 stimulation with or without addition of ILC3s. Foxp3YFP+ Treg cells were surface stained for CD25, CTLA4, LAG3, KLRG1, ICOS, GITR. IL-10 production was tested via intracellular cytokine staining. Similarly, CD4+ T cells were selected from human peripheral blood. ILC3s were sorted from human tonsils. Human T cells were differentiated to Tregs with TGF-β, IL-2 and CD3/CD28 stimulation in the absence or presence of ILC3 or ILC1. Foxp3, KLRG1, ICOS expression was quantified by surface staining. IL-10 and GranzymeB level was measured by intracellular staining. FINDINGS and CONCLUSION: Murine Treg cell differentiation in the presence of ILC3s were reduced significantly. However, surface expression of CTLA-4 and LAG3 in Treg cells was elevated whereas GITR, CD25, ICOS, CD25, LAP levels remained comparable in the presence of ILC3s compared to those differentiated in the absence of ILC3s. Human Treg generation in the presence of ILC3s was comparable to the condition without ILC3s. Importantly ILC1 addition significantly improved the yield. Presence of human of ILC3s in the Treg cultures augmented KLRG1 expression by Treg cells. Presence of ILC3s during Treg cell generation impacts the expression of surface proteins associated with the suppressive machinery of Treg cells in humans and mice.
PROTEIN-ENGINEERED MOLECULES CARRYING GAD65 EPITOPES AND TARGETING CR1 SELECTIVELY DOWN-MODULATE DISEASE-ASSOCIATED HUMAN B LYMPHOCYTES

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Introduction: Type 1 diabetes mellitus is an autoimmune metabolic disorder characterized by chronic hyperglycemia, a result from progressive immune-mediated destruction of insulin-producing beta-cells of the pancreas. B cells are critical players in the pathogenesis of the disease as modulators of the immune response via autoantibodies and cytokines production and T cell activation. A membrane bound enzyme on the pancreatic beta-cells, GAD65, is the main autoantigen in type 1 diabetes. Autoantibodies against GAD65 mediate the beta-cells destruction and decline of pancreatic functions. The human complement receptor type 1 (CR1) on B- and T-lymphocytes has a suppressive activity on these cells.

Purpose: We hypothesized that it may be possible to eliminate GAD65-specific B cells from type 1 diabetes patients by using chimeric molecules, containing an anti-CR1 antibody, coupled to peptides resembling GAD65 B/T epitopes. These molecules are expected to bind selectively the anti-GAD65 specific B-cells by the co-crosslinking of the immunoglobulin receptor and CR1 and to deliver a suppressive signal.

Method: 3D9 antibody specific to the negative receptor CD35 was obtained using the hybridoma technology. Two synthetic peptide epitopes derived from GAD65 protein, and the anti-CD35 monoclonal antibody were used for the construction of two chimeras. The peptides were chosen as possible epitopes of HLA class II proteins using EpiDOCK server. The chimeric molecules were constructed by separate conjugation of peptides to anti-CD35 antibodies using the zero-length crosslinking agent EDC. For the initiation of the protein conjugation, a lysine carrying Ahx linker was introduced to the C-end of the peptides during the peptide synthesis. The immunomodulatory activity of the engineered antibodies was tested in vitro on FACS analyses, ELISA, ELISpot and proliferation assay using PBMCs from diabetes patients and healthy individuals as a control.

Results: The constructed chimeric molecules are able to bind specifically the population of autoreactive B lymphocytes. It was proved that they can modulate selectively the activity of GAD65-specific B-lymphocytes and the production of anti-GAD65 IgG auto-antibodies by co-crosslinking of the inhibitory CR1 and the BCR. A reduction in the number of anti-GAD65 IgG antibody-secreting plasma cells and increased percentage of apoptotic B lymphocytes was observed after treatment of PBMCs from patients with type 1 diabetes with engineered antibodies. While no such effect was detected for the healthy controls.

Discussion: The present manuscript explores a novel approach for suppression of disease-associated B-lymphocytes during T1DM conditions. We demonstrate the use of a protein-engineered chimeric molecule to down-regulate GAD65-associated autoreactive B cells and thus provide a possible specific therapy for human autoimmune diabetes.
Introduction: Systemic Lupus Erythematosus (SLE) is a polygenic autoimmune disorder affecting multiple organs and systems. Hormones can influence the outcome of the disease which is especially evident as more females are predisposed to autoimmunity. Thus it is urgent to address the question how SLE can influence female fertility. Pristane-induced mouse model of SLE is very suitable for studying the disease especially in account of the healthy animals with the same background that can serve as a control. Purpose: To follow the effect of SLE manifestations on the production and development of mouse oocytes.

Method: Lupus-like symptoms were induced through intraperitoneal injection of hydrocarbon oil pristane in non-autoimmune Balb/c mice. Flow cytometry was used for the detection of CD25/CD69 activation markers and apoptosis assay. The levels of cytokines, autoantibodies in the sera and the number of autoantibody-producing plasmocytes were quantified by ELISA, protein array and ELISpot. The oocytes were collected from the oviducts after hormonal stimulation or from the ovaries for in vitro maturation. A couple of specific markers were chosen in order to determine the normal development of the oocytes and follicles: chromatin, tubulin and actin structures. For their detection were used Hoechst 33258, FITC-labeled alpha-tubulin antibody and rhodamine-labeled phalloidin, respectively.

Results: A single i.p. injection of pristane leads to the development of the typical SLE symptoms such as production of different autoantibodies accompanied by massive glomerular depositions of IgG-containing immune complexes in the kidneys, and proteinuria. The total number of obtained metaphase oocytes from lupus mice was significantly lower compared to healthy controls. The maturation rate, i.e. the proportion of eggs reaching metaphase II, was also lower for lupus mice compared to control animals. For each oocyte, four characteristics were described – spindle morphology, actin cap, chromosomal condensation and alignment. Many specific abnormalities in the lupus group were found, including long chromosomes, disorganized spindle and missing actin cap.

Discussion: Pristane-induced mouse model of lupus exhibited numerous impairments of the reproductive system which may result due to disease activity, autoantibodies or damage in molecular mechanisms through the process of reproduction.
FUNCTIONAL ELIMINATION OF AUTOREACTIVE T AND B CELLS BY ANTI-ANNEXIN A1 ANTIBODY THERAPY IN MRL/LPR MURINE MODEL OF SYSTEMIC LUPUS ERYTHEMATOSUS

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Introduction: Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease characterized by a broad range of pathological manifestations. The clinical picture of SLE is heterogeneous, affecting heart, joints, skin, lungs, blood vessels, liver, kidneys, and nervous system. In SLE, tissue damage in multiple organs is caused by autoantibodies and the resulting immune complexes. Annexin A1 (ANXA1) also known as macrocortin, renocortin and lipocortin-1 is a protein that binds to phospholipids in a Ca2+ dependent manner. As endogenous modulator of the immune system Annexin A1 plays a dual role in both innate and adaptive immune cells. It effects many physiological processes, including cell growth, differentiation, membrane fusion, endocytosis and exocytosis.

Purpose: In the present study we have investigated in vitro and in vivo the possibility to modulate the autoimmune response in MRL/lpr mouse model of systemic lupus erythematosus using an anti-AnxA1 antibody.

Method: Groups of lupus-prone MRL/lpr mice were treated with an anti-annexin A1 monoclonal antibody and the disease activity and survival of the animals were monitored. ELISA and ELISpot assays, RT-PCR, cell proliferation assay, flow cytometry, histological and immunofluorescence kidney analyses were used to determine the levels of cytokines, anti-dsDNA antibodies and kidney injuries.

Results: Either CD3+ or CD19+ MRL/lpr lymphocytes exhibited surface expression of ANX A1. Incubation of anti-CD3/CD28 stimulated spleen cells from MRL/lpr mice with different concentrations of the anti-ANX-A1 antibody exhibited a significant dose-dependent decrease of CD25+/CD69+ cells and inhibition of T-cell proliferation. The treatment significantly reduced the number of IgG anti-dsDNA antibody producing plasma cells and increased the percentage of phosphatidylserine expression within CD3-gated and CD19-gated cells. The in vivo treatment with the anti-ANX A1 antibody resulted to a suppression of dsDNA-specific B cells. Administration of monoclonal antibody prevented the appearance of high levels anti-dsDNA IgG antibodies in young (7 week-old) MRL/lpr mice. In addition, the 7 week-old animals treated with the anti-ANX A1 antibody were protected from kidney mononuclear cell infiltration, pathological glomerular immune-complex deposition and enlargement of the peripheral lymph nodes and spleens.

Discussion: Several approaches have been used to investigate the pathogenetic contribution and selective elimination of B and T cells in lupus-prone MRL/lpr mice which develop disease symptoms very close to the abnormalities observed in human SLE. Using intact MRL/lpr mice we have successfully suppressed dsDNA-specific B cells and lupus progression, prevented the appearance of autoantibodies and significantly prolonged the survival.
INVESTIGATION OF CHANGES IN EXOSOMES PROFILE DURING STORAGE PERIOD OF ERYTHROCYTE SUSPENSIONS

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Introduction: Exosomes are structures of 0.03-0.1 µm which are derived from the cell membrane, which are thought to play a role in the intercellular communication, signal transduction, transfer of genetic material and regulation of the immune response.

Purpose: The aim of this study was to investigate the relationship of exosomes in erythrocyte suspensions (ES) with leukoreduction and storage period. In this study, we expected to obtain new information about the origins of the immunomodulatory effects of allogeneic blood transfusion (TRIM).

Material-Method: Our study was carried out with the approval of the Research Ethics Committee of Bursa Uludağ University Medical Faculty (2017-1 / 47). ES’s prepared from whole blood units taken from ten blood donors and were divided into two equal parts. Leukoreduction was applied to one part of the two equal blood components, therefore leukoreduced ES’s (LR-ES) were obtained. Other part of the two equal blood components were accepted as non-leukoreduced ES (NL-ES). Each two group were divided four equal part for 0, 14, 28 and 42 storage day samples. Exosomes were isolated from each sample via exosomes purification kit (Norgen, ON, Canada) at the related storage days. Obtained exosomes were stored in deep freeze (–80°C). Flow-cytometric (FC) analyses was performed for exosome characterization and to measure their levels. The steps of FC analysis were as followed: 1. Carboxyl latex beads (LifeTechnology, Eugene, OR, USA) were coated with anti-CD9 antibody (Bio Legend, San Diego, CA, USA). 2. The quantity of exosome in samples was calculated with BCA Protein Assay (Pierce, Thermo Scientific, Rockford, IL, USA). 3. Exosomes were conjugated to carboxyl latex beads with proper volumes and quantity. 4. Exosome-bead conjugates were labeled with specific monoclonal antibody and were analyzed with FC (Navios, Beckman Coulter, Indianapolis, IN, USA).

Results: Exosomes which were derived from Th, NK, NK-T cells were not detected in our study. There were no significant differences in exosome profiles between NL-ES and LR-ES groups. Statistically significant results (in comparison with 0th day) were found as below. Exosomes which were derived from; • T lymphocytes, decrease in LR-ES groups at 42nd-day samples (p<0.05) • Tc lymphocytes, decrease in NL-ES groups at 14th day and 42nd-day samples (p<0.05) • B lymphocytes, decrease in NL-ES groups at 28th day and 42nd-day samples (p<0.05) • MDSC and G-MDSC, decrease in both NL-ES groups and LR-ES groups at 14th day, 28th day and 42nd-day samples (p<0.05). Despite these decreasing trend, these exosomes levels were found clearly high at the 42nd day samples.

Discussion: Exosomes which are derived from MDSC and G-MDSC, which have immunosuppressive property can play an important role in TRIM development. Although the effects of transfused exosomes on the recipient immune system are unknown, this issue is open to new investigations. In vivo studies that are going to be planned for this purpose might expose valuable findings. Our study also shows that exosomes in erythrocyte suspensions are not affected by leukoreduction procedure. Exosomes which can freely pass from leukocyte filters may be the main cause of the insufficiency of leukoreduction to prevent TRIM.
DOES SOLUBLE SLAMF7 CONCENTRATION QUANTIFICATION CAN BE USED AS A DIAGNOSTIC TOOL FOR IGG4-RELATED DISEASE?

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Title: Does soluble SLAMF7 concentration quantification can be used as a diagnostic tool for IgG4-related disease? 

Introduction: IgG4-related disease is a multiorgan, chronic, fibro-inflammatory disorder. Its diagnosis is made by biopsy and measuring serum IgG4 levels. But IgG4 levels can give false results sometimes. Although its pathophysiology hasn’t been clearly elucidated yet, it is believed that CD4+ SLAMF7+ cytotoxic T cells in affected tissues drive the disease process.

Purpose: Our aim is to quantify soluble SLAMF7 concentration in serum and to see whether it correlates with the disease state. Methods: Soluble SLAMF7 concentration is measured in human plasma samples from 38 healthy donors, 40 systemic sclerosis patients, 40 idiopathic pulmonary fibrosis patients and 42 IgG4-related disease patients by using ELISA kits for SLAMF7. All p-values were determined by Mann-Whitney test.

Results: SLAMF7 concentration is highest in IgG4-related disease group compared to other 3 groups. Systemic sclerosis and healthy donors group have lowest results in a close range. P value = 0.002 between IgG4-related disease and and healthy donors.

Discussion: Soluble SLAMF7 concentration is highest in IgG4-related disease among 4 groups. We propose that we can use SLAMF7 quantification in serum as a diagnostic tool for IgG4-related disease. In next studies we are going to investigate whether this concentration correlates with CD4+ SLAMF7+ T cell numbers to see if we can have an assumption about disease activity and prognosis.
EVALUATION OF NEW IL-12 FAMILY (IL-35 AND IL-39) MEMBERS IN DIFFERENT FORMS OF BRUCELLOSIS

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Backgrounds: As one of the important public health problem the Brucella infection would activate the immune system. The outcome of Brucella infection was affected significantly by the interactions between Brucella and host immune response. Cytokines play important role in anti-bacterial immunity. The recently identified cytokines have been described for their immune-modulating actions in numerous inflammatory diseases, autoimmune disorders and infectious diseases. Several of these are new members of cytokine superfamilies, including several IL-12 superfamily member (IL-35, IL-39). IL-35 is a anti-inflammatory cytokine is secreted by activated macrophages, dendritic cells, B cells and FoxP3+ Treg cells. IL-39 is a pro-inflammatory cytokine is secreted by activated B cells. The aim of this study was to investigate the role of these new immunomodulatory cytokines in Brucella infections.

Methods/Results: Serum IL-35 and IL-39 concentrations in 40 acute, 40 chronic brucellosis patients and 40 healthy control subjects were analyzed by ELISA. The mRNA levels of IL-35 and IL-39 in PBMCs were determined by RT-qPCR. Both serum IL-35 and IL-39 concentrations were significantly higher in healthy controls than in brucellosis patients and IL-35 and IL-39 serum levels of chronic brucellosis patients were higher than those of acute cases (p<0.05). Furthermore, the expression of Ebi3/IL-12A (IL-35 genes) and Ebi3/IL-23A (IL-39 genes) were found to be downregulated in acute brucellosis patients compared with the healthy controls, whereas they were upregulated in chronic brucellosis patients. In addition, the expression levels of Ebi3/IL-12A and Ebi3/IL-23A genes in acute brucellosis patients were lower than chronic brucellosis patients. Despite all these results, no statistically significant difference was found in terms of gene expressions.

Conclusion: Our study demonstrated that IL-35 and IL-39 may be novel markers associated with different forms of brucellosis. Taken together, the significantly higher concentrations of IL-35 in chronic patients than in acute patients infer that this cytokine might be play role in the suppression of the immune response against brucellosis and its progression to chronicity.
NOVEL IL-1 FAMILY (IL-36, IL-37 AND IL-38) MEMBERS AS POTENTIAL BIOMARKERS FOR CHRONIC BRUCELLOSIS

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Backgrounds: Brucellosis is a common disease over the world. Although there are different forms of brucellosis, its mechanism from acute form to chronicity has not been fully understood yet. Bacterial infections usually trigger strong innate immune mechanisms, including IL-1 cytokine secretion. The newer members of the IL-1 family, IL-36, IL-37 and IL-38 have immunomodulatory effects and play important role in immune responses. IL-36 is a pro-inflammatory cytokine which mediates a lot of infectious diseases. IL-37 and IL-38 are anti-inflammatory cytokines which reduce inflammation and suppress immune responses. The aim of this study was to investigate the role of these new immunomodulatory cytokines in Brucella infections and its transition from acute to chronic form.

Methods/Results: Serum IL-36, IL-37 and IL-38 concentrations in 40 acute, 40 chronic brucellosis patients and 40 healthy control subjects were analyzed by ELISA. The mRNA levels of IL-36, IL-37 and IL-38 in PBMCs was determined by RT-qPCR. There was no significant difference in serum IL-36 levels among the groups. Both serum IL-37 and IL-38 concentrations were significantly higher in healthy controls than in brucellosis patients. IL-37 and IL-38 serum levels of chronic brucellosis patients were higher than those of acute patients. Furthermore, the expression of IL-36G (IL-36 gene) and IL-37 (IL-37 gene) were found to be downregulated in both acute and chronic brucellosis patients compared with the healthy controls. Contrastly, IL-1F10 (IL-38 gene) expression was found to be upregulated in healthy controls. The expression levels of IL-37 and IL-1F10 genes in acute brucellosis patients were higher than chronic brucellosis patients but IL-36G were lower. Despite all these results, no statistically significant difference was found in terms of gene expressions.

Conclusion: The current data suggested that serum concentrations of IL-37 and IL-38 may be diagnostic markers of brucellosis. Taken together, the significantly higher concentrations of IL-37 and IL-38 in chronic patients than in acute patients infer that these cytokines may be involved in the suppression of the immune response against brucellosis and its progression to chronicity.
Introduction: Probiotics have immunomodulatory properties and recent research focuses on identification of immunoregulatory probiotic molecules. Studies point out the involvement of Toll like receptors (TLR) in probiotic action (1). Several bacterial TIR domain proteins were shown to alter TLR signaling and change the immune response. These proteins structurally mimic and can bind to the human adaptor TIR domain proteins (2,3). AIM Here we propose that probiotics also produce proteins with TIR domain structure which can interact with MyD88 and TIRAP. Bioinformatics search revealed the presence of putative TIR domain protein genes in the genomes of Bifidobacterium breve and Lactobacillus casei. Multiple sequence alignments of B. breve TIR domain protein (BbTIR) and L. casei TIR domain protein (LcTIR) with known human and bacterial TIR domain proteins verified the presence of conserved TIR domain signature regions. The aim of this study is to clone and purify BbTIR and LcTIR in order to test their interactions with human TIR domain proteins.

Method: Probiotic TIR domain genes were cloned in fusion with N-terminal his tags. Human TIR domain adaptors MyD88 and TIRAP were cloned into pGEX-4T2 to obtain GST fusion proteins. Recombinant proteins were purified by affinity chromatography with Cobalt or Glutathione resins. The in vitro interaction of proteins were tested by GST pull down assay. 150 μg of 6his-BbTIR was mixed with 150 μg of GST, GST-MyD88 or GST-TIRAP, diluted in buffer A (50 mM Tris, 150mM NaCl, 5% glycerol, 1mM EDTA and 5mM DTT), incubated 30 min in ice with mild shaking before addition of 300 μL of Glutathione bead slurry. The mixture were incubated for 2 hours in ice and loaded to spin columns, flow through collected and washed with buffer A. The total protein bound to resin was eluted by boiling the resin in SDS-PAGE sample buffer and samples were analyzed by SDS-PAGE and Coomassie brilliant blue R250 staining and with polyHistidine antibody after western blotting.

Findings And Conclusion: The structures of BbTIR and LcTIR were modelled and the models support the presence of the TIR domain fold. BbTIR was expressed and purified. LcTIR, on the other hand, was toxic to E.coli cells and was poorly expressed. A possible interaction between BbTIR and human MyD88/TIRAP was investigated using an in vitro GST pull down assay. The results proved the interaction; as BbTIR was pulled down on glutathione resin in the presence of GST-MyD88 and GST-TIRAP and not GST alone. This study is the first to show that probiotics produce functional TIR domain proteins which interact with human adaptors. In future studies, we plan to focus on structural characterization of BbTIR using SAXS and macromolecular crystallography. Overall, these studies will provide evidence, for the first time, that probiotics have structurally and biochemically functional TIR domain proteins.


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STRUCTURAL ANALYSIS OF POLYMERIZATION DYNAMICS OF ASC SPECK THROUGH ITS PYD AND CARD DOMAINS

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Aim: Apoptosis-associated speck-like protein containing a CARD (ASC) is a 22 kDa protein containing conserved PYD and CARD domains that belongs to the death-fold superfamily. Death folds have been shown to consist of six anti-parallel alpha-helical domains. The homotypic interactions between one death-fold domain with another occurs via certain helices, providing at least three surfaces of contact (Type I, Type II and Type III). ASC protein is a key player in inflammasome formation having adapter role between receptor and effector proteins. ASC also has the ability to form supramolecular globular complex called the ASC speck through PYD and CARD homotypic interactions. When expressed in truncated form as PYD and CARD separately, these domains form fiber-like polymeric structures. Apparently, these fibers compact onto each other during the wild type ASC polymerization. Our aim is to elucidate the importance of specific locations on PYD-PYD and CARD-CARD homotypic interaction surfaces during the polymerization process.

Method: PYD and CARD domains of ASC protein were cloned into pEGFP and pmCherry plasmids to have EGFP and mCherry tagged PYD and CARD domains separately. PCR-based site-directed mutagenesis method was used to create certain single mutations such as E13A, D48A, Y60A, E130A, Y146A, R150A, M159A on the domains and double-mutant combinations were designed and introduced at evolutionary important aminoacid residues to hit one and/or two interaction surfaces in same time. Effects of the mutations were visualized using fluorescence and laser-scanning confocal microscopy and qualified upon change in level of organization of the phenotype from filamentous to soluble. Next, we analysed homotypic PYD-PYD and CARD-CARD interactions using Förster Resonance Energy Transfer (FRET) technique for quantification of interaction rate upon presence of the different disruptive mutations. We also applied Atomic Force Microscopy to analyse change in interaction strength between wild-type and mutant CARD domains.

Findings: Our results show that PYD mutants, which’ve been known to disrupt homo-oligomerization, are able to provide multimeric filamentous structures when expressed together with their wild-type counterparts although CARD mutants have less tolerance to aminoacid changes in the interaction surfaces. We identified certain mutations that increase, decrease or block FRET signal. Our Atomic Force Microscopy (AFM) based Single Molecule Force Spectroscopy (SMFS) analysis on both of wild-type and E130A mutant of CARD provide quantitative result about how physical strength of the homotypic CARD interactions change upon disruption of Type I interaction surfaces of death-fold domains.

Conclusion: Our study will provide better explanation and new insights about the oligomerization dynamics and interaction strength of inflammasome complex through death domains.
COMPARISON OF MITOGEN-INDUCED LYMPHOCYTE PROLIFERATION IN HEALTHY PEDIATRIC AND ADULT AGE GROUPS

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Introduction: The proliferation of lymphocytes, the initial phase in acquired immunity is important for competence of immune cells. Proliferation tests both in immunologic research and immune diagnosis is gaining attendance day by day. This test is used especially for patients with primary immune deficiencies and also for monitorization of immune responses following bone marrow transplantation. Healthy controls are a requisite of comparison of responses of patients, while utilization of adult healthy individuals as controls for pediatric patients is a question. Purpose: The aim of this study was the investigation and comparison of mitogen induced proliferation responses of peripheral blood mononuclear cells (PBMCs) and T- and B-lymphocytes in adult and pediatric healthy individuals.

Method: Nineteen pediatric and 20 adult healthy individuals were enrolled in this study. PBMCs isolated from peripheral blood samples of donors by ficoll gradient centrifugation were initially labelled with CFSE and were cultured for 120 hours with the absence and existence of polyclonal activators; phytohemaglutinin (PHA) and anti-CD2, anti-CD3 and anti-CD28 monoclonal antibody cocktail (CD-Mix). Following termination of the cell culture, PBMCs were stained with antibodies against CD4 and CD19 surface molecules which mark T cells and B cells, respectively. Proliferation percentages of CD4+ T cells and CD19+ B cells together with total PBMCs were determined by flow cytometry.

Results: Findings obtained from this study revealed similarity between pediatric and adult age groups with respect to PBMCs, T- and B-cells responses to mitogen stimulation. The only alteration observed was a significantly high proliferation of pediatric CD4+ T cells in response to PHA stimulation. Pediatric individuals were distributed into age groups of 0-2 years, 3-5 years and 6-18 years, and no differences of mitogen-triggered proliferative responses were observed among age groups.

Discussion: The findings of this study revealed similarity between pediatric and adult healthy controls. These findings may suggest the possible utilization of adult controls for comparison with pediatrics.
IN THE PURSUIT OF THE NLRP7’S INTERACTION PARTNERS: WHAT IS THE ROLE OF AN INNATE IMMUNE RECEPTOR IN ONCOGENESIS?

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Introduction And Aim: Inflammasomes are key multi-protein complexes formed upon cellular infections or stresses and regulate inflammation-associated signaling by assisting secretion of pro-inflammatory cytokines, interleukin (IL)-1β and IL-18. Nucleotide-binding domain and leucine-rich repeat-containing family, pyrin domain-containing 7 (NLRP7) is one of the innate immunity proteins and found in the activation of proinflammatory caspases by participating inflammasome oligomerization. Although there is little known about NLRP7 except for the fact that it is an inflammasome component, its expression levels increase in testicular seminoma and endometrial cancer cell lines in comparison with healthy control groups, therefore, we hypothesized that NLRP7 may have an oncogenic role.

Method: To investigate the oncogenic role of NLRP7, tumor xenograft experiment was performed by injecting human endometrial carcinoma cells which stably express NLRP7 and control cells subcutaneously in mice. Upon obtaining tumor data, liquid chromatography-mass spectrometry applied after pulling down of NLRP7 from Hec1a cells to elucidate the molecular mechanism behind the contribution of this protein to oncogenesis. To detect the most robust and important interactions, results were normalized using intensity-based absolute quantification (iBAQ) and the list of candidates was narrowed down. Three potential NLRP7 interactors, which are categorized in three groups in pathway analysis, were picked out for further validation based on their fold changes. As a further step, to investigate how NLRP7 contributes tumorigenesis, knockout Hec1a cell line was generated using CRISPR/Cas9 technology. Now, phenotypic assays are ongoing progress in our laboratory.

Findings: Our results indicate that NLRP7 overexpression enhances tumor growth in mice compared with that of the control group. As a second finding, the molecular mechanism of cancer, DNA damage, and remodeling of epithelial adherens junctions was predominant among the highlighted protein networks. In addition to that the network analysis showed that NFB and ERK pathways are center of it. Our results showed that one of our putative interaction partners Brat1, a DNA damage repair protein, interacts with NLRP7 in Hek293 cells. Rabl and Rab5a, two different GTPase proteins, was chosen as second and third putative interaction partners. The interaction between NLRP7 and these proteins are transient and only form tight complexes when GTP bound. Therefore, constitutively active Rabl effector domain (G23V) and Rab5a effector domain (Q79L) was generated by Side-Directed Mutagenesis. Indeed, our findings showed that both interactions significantly increased when CO-IP was performed using their mutant forms.

Conclusion: In our study, we confirmed three novel interactors of NLRP7 by co-immunoprecipitation. Our findings might provide a deeper understanding about how the dysregulation of an innate immune receptor protein 'NLRP7' may drive oncogenesis by disrupting several essential processes including cellular proliferation, cell death and DNA damage response.
Introduction: Nod like receptor family pyrin containing protein 7 (NLRP7) belongs to the family of cytosolic pattern recognition receptors, which is contributing to formation of inflammasome complexes in order to regulate immune responses. However, the research on the protein is mainly based on the genetic background of its non-inflammatory role in trophoblast differentiation and it has been shown as a causative gene for hydatidiform mole, gestational trophoblastic disease. Therefore, current understanding of mechanism and regulation of inflammasome is mostly enlightened by NLRP3 studies whereas various aspects of NLRP7 inflammasome remain to be elucidated up to now. Recent studies showed microbial acetylated lipopeptides are specifically sensed by NLRP7, leading to ASC-dependent caspase-1 activation and maturation of IL-1β and IL-18. Furthermore, it has been also revealed that Mycoplasma spp., Staphylococcus aureus and Listeria monocytogenes can initiate formation of NLRP7 inflammasome even though previous studies stated NLRP7 suppresses IL-1β processing.

Aim In the study it was aimed at clarifying how NLRP7 inflammasome complex forms. METHOD Co-IP with inflammasome components IF for colocalization of inflammasome components with NLRP7 Knockdown of NLRP7 in THP1 cells via CRISPR-Cas9 Technology ELISA to detect IL-1ß secretion from THP1-NLRP7OE cells increases upon P. aeruginosa infection. Cytokine profiling of THP1-NLRP7OE cells upon P. aeruginosa infection

Findings According to our study, overexpressed NLRP7 is colocalized with caspase-1 and ASC in inflammasome complex and thus IL-1ß secretion increases. The interaction of NLRP7 with caspase-1/5 and ASC was showed by co-IP studies. Moreover, it has been observed that upon Pseudomonas aeruginosa infection, NLRP7-overexpressing THP-1 cells secrete more IL-1ß as well as GM-CSF, TNF and IL-6 while IL-1ß level is decreasing when NLRP7 is downregulated by CRISPR/Cas9 technology. Our results contribute to the clarification of NLRP7 inflammasome signaling.

Conclusion: 1. NLRP7 can form inflammasome-like structure together with ASC and procaspase-1, resulting in caspase-1 activation, which, in turn, leads to IL-1ß secretion. 2. Upon P. aeruginosa infection IL-1ß secretion is higher in THP1 cells overexpressing NLRP7 than WT THP1 cells. 3. NLRP7 is required for P. aeruginosa driven IL-1ß secretion and pro-IL-1ß expression. 4. Upon Pseudomonas aeruginosa infection, NLRP7-overexpressing THP-1 cells secrete more IL-1ß as well as GM-CSF, TNF and IL-6
Introduction: Monocyte-to-macrophage differentiation is simultaneously induced ex vivo and regulated according to cytokine milieu and matrix adhesion. However, depending on the microenvironment conditions, these cells display both phenotypic and functional heterogeneity. This study evaluates the helper T (Th) cell costimulatory capacity of the programmed cell death ligand 2 (PD-L2)-expressing macrophages found in suspension in the serous fluids drained from surgical wound zones.

Methods: The fluid draining from the surgical wounds of patients who underwent minor surgery [n=14 (10 females, 4 males), median age 45 (min 31 - max 58)] or major surgery [n=18 (12 females, 6 males), median age 58 (min 47 - max 73)] was collected under sterile conditions. Peripheral blood was obtained from healthy donors who did not have inflammatory diseases. Peripheral blood mononuclear cells (PBMCs) and the leukocytes found in the drainage fluid were separated by density gradient. CD14, CD68, CD80, CD86, CD206, CD163, PD-L1, PD-L2 markers were used to analyze the macrophage population by flow cytometry and immunofluorescence. Co-cultures were established between helper T (Th) cells and the macrophages wherein PD-L2 was also blocked by a neutralizing mAb; then, T cell proliferation and IFN-γ secretion was assessed by flow cytometric eFluor670 dilution assay and ELISA, respectively.

Results: A prominent sub-population of CD14+ cells, which was found in the surgical drainage fluid, was determined as the adhesion- independent CD68+ macrophages with high PD-L2 expression. These macrophages displayed a heterogeneous phenotype in terms of CD163 and CD206 expressions related to M2 phenotype. Especially, macrophages expressing CD206 had higher PD-L2 levels. Intriguingly, these CD206+CD163+PD-L2hi cells were also highly positive for CD80 and CD86 M1-related markers. These cells had only limited capacity to induce Th cell proliferation and IFN-γ secretion which were restored upon PD-L2 blockade.

Conclusion: High and constitutive expression of PD-L2 on the macrophages from surgical wound zones both displayed M1 and M2 like features; however, blockade of PD-L2 co-inhibitory molecule supported generation of IFN-γ secreting Th1 subset. Therefore, PD-L2+ macrophages may function as a safety measure that reins acceleration of Th1 responses to avoid exacerbation of inflammation in the surgical wound zone.

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Introduction: Myeloid-derived suppressor cells (MDSCs) are of heterogeneous regulatory immune cells that arise in response to chronic inflammation. In the mouse tumor the amount of MDSCs are especially found to be increased both in the peripheral blood and spleen. Since spleen samples are not easily accessible in cancer patients, MDSCs are generally obtained from blood. This study aims to evaluate and characterize the MDSC subsets found in the spleen of pancreatic and gastric cancer patients.

Methods: Spleen and peripheral blood samples of 17 gastric cancer patients and 9 pancreatic cancer patients who underwent pancreatectomy or gastrectomy were collected. Blood samples from 30 healthy individuals were used as controls. Splenocytes, which were prepared in suspension, and peripheral blood were layered over 1.077 g/ml Ficoll and 1.119 g/ml Ficoll. The expression of CD11b, CD33, CD66b, HLA-DR, CD14, CD15, LOX1, and CD16 were tested by flow cytometry. CD11b+CD33dim/moCD66b+ cells in the 1.077 g/ml phase were purified with magnetic MACS followed by FACS. In order to measure their immunomodulatory character, these cells were co-cultured with PBMCs obtained from healthy donors under anti-CD3 stimulation and T cell proliferation and IFN-γ secretion were tested. The cytological features of spleen-derived MDSCs were also evaluated by May-Grünwald Giemsa staining.

Results: CD11b+CD33dimCD66b+ cells (PMN-MDSCs) were the most prominent MDSC sub-population both the peripheral blood and spleen of the patients. However, the ratio of immature myeloid cells (especially, metamyelocytes and myelocytes) was higher in the spleen. The amount of CD11b+CD33brightHLA-DRlo/-CD66b-cells (M-MDSCs) and CD11b+CD33+HLA-DR-CD66b- (e-MDSCs) cells were also augmented in the cancer patients' peripheral blood and spleen.

Conclusion: Our results indicate that the human spleen may serve as a potential reservoir for immature myeloid cells with high immune modulatory capacities in cancer.

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CD55 DEFICIENT PATIENT DERIVED EXOSOME-DEPENDENT IMMUNE ACTIVATION BEFORE AND AFTER ECULIZUMAB THERAPY

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CD55 or Decay-accelerating-Factor (DAF), is a membrane bound protein which inhibits classical and alternative complement pathways. Deficiency of CD55 has been recently characterized as hyperactivation of complement, angiopathic thrombosis, and protein-losing enteropathy (CHAPLE Syndrome). Herein, the breadth of immune dysregulation of CD55 deficient PBMC in response to endosomal and cytosolic sensor ligands stimulations were explored. Immune responses were investigated before (BT) and after (AT) single dose of Eculizumab therapy.

Freshly isolated BT and AT PBMCs of 4 diagnosed CHAPLE individuals were stimulated with endosomal TLR (p(I:C), R848, D35 CpG ODN) and cytosolic PRR ligands (transfection of cGAMP, p(dA:dT), LPS) for 24 hours. After stimulation, supernatants were collected, and responses were assessed by cytokine ELISA. Both BT and AT PBMCs that were stimulated with endosomal TLR ligands or transfected with cGAMP, p(dA:dT) and LPS secreted less IL-1β, IL-6, IFN-α and IP-10 compared to healthy PBMCs. Moreover, BT and AT PBMCs secreted less IL-10 upon LPS transfection and R848 stimulation.

Exosomes from BT and AT plasmas were isolated and incubated with BT and AT patient PBMCs. After 24 hours, supernatants were collected, and cytokine secretion levels were determined by ELISA. When BT or AT exosomes were incubated with Before therapy patient PBMCs, we observed that AT exosomes induced “>2-fold less” secretion of IL-1β, TNF-α and IFN-γ cytokines while inducing “2-fold more” IL-10 production compared to BT exosome. When after therapy patient PBMCs were incubated either with BT or AT exosomes, similar results as described above were recorded. AT patient immune cells secreted less amounts of cytokine in response to AT exosome treatments. In separate experiments, BT and AT exosomes were incubated with two health individuals’ PBMCs. Results revealed that PBMCs that were incubated with AT exosomes secreted less IP-10 compared to induction mediated by BT exosomes.

Our findings suggested that after a single dose of Eculizumab therapy PBMCs displayed lesser pathologic immune responses. Of note, the endosomal and cytosolic PRR responses of patient immune cells were lower and did not return to healthy donor levels. Furthermore, AT but not BT circulating exosomes mediate less severe inflammatory response.

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NEUTROPHILS REINFORCE CYTOTOXIC T CELL RESPONSES IN A CO-CULTURE MODEL MIMICKING TUMOR MICROENVIRONMENT IN LUNG CANCER

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Introduction: Cytotoxic T lymphocytes (CTL) are critical mediators in anti-tumor immunity. Neutrophil granulocytes have been shown to dominate the immune landscape of the non-small cell lung cancer (NSCLC). Immune regulatory role of neutrophils has not yet been fully elucidated. Hence, this study aims to evaluate CTL responses in the presence of neutrophils, monocytes and lung adenocarcinoma cells, in vitro.

Methods: Peripheral blood CD66b+ neutrophils, CD14+ monocytes, and CD8+CD4-CD56- T cells were purified from the healthy volunteers with density gradient centrifugation, followed by MACS and/or FACS. At different ratios, CTLs were co-cultured with freshly isolated monocytes and/or neutrophils and NSCLC cell lines (A549, NCI-H1299, or NCI-H441). Soluble anti-CD3 mAb was added in order to test the antigen-independent influences on CTLs. Proliferation, viability and activation of immune cells were tested by flow cytometry. Reactive oxygen species (ROS) production capacity of neutrophils in different co-culture models were measured by flow cytometry.

Results: The presence of lung cancer cells and monocytes prolonged the survival of neutrophils. In co-cultures, neutrophils swiftly acquired an activated state with decreased CD62L and CD66b; nevertheless, ROS production was decreased. The presence of monocytes, neutrophils, and lung cancer cells augmented CTL proliferation and expression of TIM-3, PD-1, LAG-3, CTLA-4 co-inhibitor receptors. Under certain conditions lacking monocytes as major supporters of T-cell proliferation, the presence of neutrophils together with lung adenocarcinoma cells, barely supported CTL responses.

Conclusion: In a co-culture setup employing neutrophils, monocytes, CTLs and lung cancer cells, which was established to mimic the tumor microenvironment, our initial findings indicate the importance of neutrophils as important modulators of CTL responses.

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ANALYSIS OF THE FREQUENCY AND ABSOLUTE NUMBERS OF ILC2 CELLS IN NON-SMALL CELL LUNG CANCER

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Introduction: Innate lymphoid cells (ILCs) are a newly discovered family of cells involved in immunity at mucosal barrier surfaces open to the exterior, which can modify immune responses by being one of the prompt responders to tissue insult. ILCs are subdivided into three groups in terms of cytokine expression, termed ILC1, 2 and 3. ILC2 cells were the first ILC group identified in the lung. While their role in various lung pathologies such as helminth infections, allergy, or fibrosis has been extensively studied, their role in non-small cell lung cancer (NSCLC) has never been addressed.

Purpose: The purpose of the ongoing study is to determine, in correlation with cancer stage, the frequency and absolute numbers of ILC2 cells in NSCLC tissue as compared to adjacent normal lung tissue, tumor-draining lymph node and peripheral blood of NSCLC patients. We also seek to determine whether HLA-DR is expressed on ILC2 cells of lung cancer patients, as it has been reported in other lung pathologies.

Method: Tumor tissue, adjacent normal lung tissue, mediastinal lymph node and peripheral blood of NSCLC patients in stages I-III, were obtained immediately after surgical resection of primary tumors. Single cell suspensions were obtained from all samples and stained with relevant antibodies to cell surface markers for ILC2 cells and HLA-DR, followed by flow cytometry analysis.

Results: We found that the frequency of ILC2 cells was highest in tumor tissue as compared to all other tissues in patients whose tumors had not spread to draining lymph nodes. The frequency of ILC2 cells in tumor samples was highest in cancer stage 3 as compared to stages 1 and 2. However, we also found that, in patients whose cancer had spread to the draining lymph nodes, the frequency of ILC2 cells was lowest in the tumor sample and highest in the peripheral blood. Absolute numbers of cells were higher in tumor tissue as compared to normal lung, indicating a preferential accumulation of ILC2 cells in tumor, however, as opposed to frequency, absolute numbers were negatively correlated with cancer stage. Interestingly, we found that ILC2 cells from NSCLC patients did not express HLA-DR, irrespective of tissue location.

Discussion: Our results indicate that ILC2 cells preferentially locate in tumor tissue with frequencies positively correlated with cancer stage. They also indicate that ILC2 cells from NSCLC patients are unable to interact with CD4 T cells by cell-cell contact via HLA-DR. However, it is important to note that additional samples need to be analyzed in order to confirm these results. Whether ILC2 cells can influence CD4 T cells via the cytokines they produce and whether they harbor a specific pattern of cytokine secretion within the tumor that may influence cancer progression remain to be determined.
CHARACTERIZATION OF C-TERMINAL LYSINE HETEROGENEITY IN BIOSIMILAR ANTIBODIES

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Introduction: Monoclonal antibodies (Mabs) intended for therapeutic use require extensive structural characterization. Microheterogeneities observed in these large molecules may impact the stability, efficacy and safety of the drug product. These include post-translational modifications such as glycosylation, C-terminal modifications, methionine oxidation, asparagine deamidation and disulfide related modifications. They have a decisive role in batch-to-batch consistency and also in the establishment of biosimilarity to an originator molecule. C-terminal lysine processing is a common post-translational modification encountered in the recombinant production of Mabs. It is considered to occur due to the enzymatic activity of carboxypeptidases in the bioreactor. Resulting molecular variants can have one, two or no C-terminal lysines depending on carboxypeptidase level and activity.

Purpose: This study aims to identify C-terminal modifications in a biosimilar Mab by using Cation Exchange (CEX) Chromatography and Liquid Chromatography-Mass Spectrometry (LC-MS). The rationale behind this approach is that the C terminal lysine heterogeneity manifests itself in the mass and isoelectric point of the molecule. Biosimilar candidate should be comparable to the originator in terms charge variants and molecular mass profiles. In cases where different charge or mass profiles are observed, the nature of these heterogeneities must be determined.

Method: A cation exchange chromatography method was developed for this particular biosimilar candidate. Presence of positively charged C-terminal lysine residues increases the pl of the molecule and allows for a charge-based separation of two, one or no lysine bearing variants. Intact mass profiles were determined with UPLC- Xevo G2 XS- QTOF system using a C4 reverse-phase column. Depending on the expected mass of the molecule, UNIFI software assigns probable modifications to the observed mass profile. Unprocessed C-terminal lysines cause a 128 Da shift in the mass spectrum which allows for a mass-based separation. Presence of C-terminal lysines were confirmed by treating the samples with Recombinant Carboxypeptidase B which specifically catalyzes the hydrolysis of basic amino acids at the carboxy terminus. Charge variants and mass profiles before and after the enzymatic reaction were compared.

Results and Discussion: A significant level of basic variants observed in CEX chromatogram were removed after the carboxypeptidase treatment which indicates that the charge heterogeneity in this biosimilar candidate is largely caused by C-terminal lysines. An additional confirmation was obtained from the intact mass profile of the biosimilar candidate which displayed three distinct species, each with a mass shift of nearly 128 Da.
PEPTIDE MAPPING ANALYSIS WITH MASS SPECTROMETRY FOR BIOSIMILAR PRODUCTS

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Peptide Mapping Analysis with Mass Spectrometry for Biosimilar Products Pegah Zahedimaram1,2, Lolai Ikromzoda1,2, Basak Ozata1, Melike Berksoz2,3, Huriye Erdogan4, Yilmaz Capan3, Batu Erman1,2, Tolga Sutlu1 1 Sabanci Universitesi Nanoteknoloji Araştirma ve Uygulama Merkezi, Istanbul, Türkiye. 2 Mühendislik ve Doğa Bilimleri Fakültesi, Sabanci Universitesi, Istanbul, Türkiye. 3 ILKO ARGEM Biyoteknoloji Merkezi, Teknopark Istanbul, Türkiye 4Kimya Bölümü, Gebze Teknik Üniversitesi, Istanbul, Türkiye.

Introduction: In quality control of therapeutic proteins peptide mapping is used for identity confirmation of the protein and detection of posttranslational modifications. Peptide mapping starts with an enzymatic or chemical treatment of the protein to selectively cleave amide bonds between amino acid residues to yield a predictable set of peptides. Proteolytic enzymes, such as trypsin, have been proven highly useful for a variety of site-specific cleavage locations. Purpose: Peptide mapping for pharmaceutical quality control purposes differs from peptide mapping for primary structure determination in several ways: (a) The expected sequence of the expressed protein is known. (b) The major concerns are minor differences in the protein, e.g., mutations, truncations, and posttranslational modifications. (c) The whole procedure must be highly reproducible and robust. (d) Many samples have to be processed on a routine basis. Therefore, the sample preparation becomes the crucial step in the whole procedure. This study compares various sample preparation procedures for peptide mapping of a monoclonal antibody.

Method: We have optimized the protein digestion protocol using two common enzymes trypsin, that hydrolyzes only the peptide bonds in which the carbonyl group is followed either by an arginine (Arg) or lysine (Lys), and chymotrypsin that cleaves peptides in C-terminal side of hydrophobic residues. While the digestions are carried out under denaturing and reducing conditions, trypsin digestion has also been performed under denaturing, but non-reducing conditions. The peptides produced are analyzed by ultra-performance liquid chromatography, using an increasing gradient of acetonitrile in water with UV light detection at 214 nm, coupled through an electrospray interface to a high-resolution time-of-flight mass spectrometry (UPLC/ Q- TOF-MS), allowing the molecular mass determination for each peptide.

Results and Discussion: The peptide mapping resulting from reduced trypsin and chymotrypsin digestions show 97% and 96% reproducible coverage, respectively. The CDRs (Complementarity Determining Regions) of the antibody are 100% covered in trypsin digestion and the light chain is 100% covered in chymotrypsin digestion. Moreover, other amino acid modifications like glycosylations could be detected. The non-reduced trypsin digestion resulted in a 99% peptide map coverage which also identifies the cysteine residues involved in disulfide bonds. The experiments for non-reduced digestion is still going on to confirm exact number and position of disulfide bonds of the protein. This project is supported by TUBITAK 115G074.
THE EFFECT OF INTRACELLULAR IMMUNE SUPPRESSION ON LENTIVIRAL GENE DELIVERY EFFICIENCY AND DISTRIBUTION

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Introduction: Genetically modified and programmed immune system cells have a great role in cancer therapy. There are various methods to achieve gene integration into the cell genome. Among these methods, viral vectors show promising future in the field of cancer immunotherapy in terms of in vitro gene transfer. Recently, several studies on genetically modified NK cells in clinical trials showed encouraging results which indicates the importance of genetic retargeting approaches based on NK cells. It is known that NK cells are one of the members of innate immune system which participates in antiviral responses. Hence, the main challenge in this setting is the delivery of the gene of interest into the genome safely and successfully using viral vectors. Purpose: Homogenous gene delivery is desired in clinical applications of lentiviral gene deliver for reaching high efficiency and avoiding any clonal selection among the genetically modified cells. In this study, we investigated vector distribution in NK cells throughout multiple viral transduction events with different vectors.

Method: We examined lentiviral vector distribution in the presence of small molecule inhibitors to block innate immune sensor pathways. Higher gene delivery efficiency can be achieved by inhibiting different pathways, such as BX-795 which mainly inhibits TBK1/IKK ε and (5Z)-7-Oxozaenol (OXO) which mainly inhibits MAPKK pathways. Viral transductions with two different vectors with separate fluorescent genes on consecutive days were performed on NK-92 cells and the results were analyzed by flow cytometry.

Results And Discussion: The absence of inhibitors during the viral transductions causes a non-homogenous distribution of viral vector within the cell population, where the gene delivery is biased towards a certain population in cell culture. The presence of intracellular immune response suppressors during viral vector delivery provides a randomization effect on viral transduction events, thereby increasing transduction efficiency and homogenizing the distribution of the vector.
DOWNREGULATION OF NLRP11 ALTERS HUMAN T CELL RESPONSES IN CO-CULTURES WITH DAUDI CELLS

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The NLR family is a relatively newly explored family of Pattern Recognition Receptors whose roles have been studied almost exclusively in innate immunity. There are 22 known members of the NLR family in humans, four of which form a multimeric protein complex called the inflammasomes. Interestingly, the NLR family members have been shown to have associations with several diseases such as atherosclerosis, type II diabetes, obesity, Alzheimer’s disease, gout and bacterial, viral and parasite infections. Of all the members of the NLR family, NOD-like Receptor 11 (NLRP11) is expressed only in primates; yet, its cellular functions as well as the specific stimulant(s) that activate it are remain unknown. To understand whether NLRP11 forms an inflammasome complex and whether it has regulatory functions in orchestrating adaptive immune responses, we investigated its potential interactors including ASC and Caspase-1 by co-IP and determined T cells responses; respectively. We also tested both extracellular and intracellular IL1β production by ELISA and western blotting, as a maker for canonical inflammasome pathway activation. High expression of NLRP11 and expression of costimulatory molecules made Daudi cells an ideal model to use in our experiments. Given that B cells are professional Antigen Presenting Cells (APC) that interact with T cells, we co-cultured human CD4+ primary T cells with Daudi cells in vitro. 40% downregulation of NLRP11 by siRNA in co-cultures resulted in the significant reduction of Th1, Th17 responses, and an increase in anti-inflammatory response whereas did not significantly affect Th2 responses when compared with control co-cultures. In brief, our studies of NLRP11 suggest a role in regulating adaptive immune responses.
TCR-NK-92 CELLS DELAY TYROSINASE-EXPRESSING A375 MELANOMA TUMOR GROWTH IN A NOD/SCID MODEL

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Introduction: The assembly of a TCR introduced by gene delivery is a complex process which requires TCR-α and TCR-β chains to form a heterodimer that associates with the four CD3 chains. This gene delivery has a risk of pairing with the complementary TCR-β or TCR-α chains endogenously expressed by the T cell. This phenomenon called mispairing, has the potential to produce TCRs of unpredictable specificity that may cause a lethal GvHD-like syndrome in vivo. Despite the promise of TCR gene therapy, the mispairing problem constitutes a bottleneck in the development of effective and safe therapies.

Purpose: Using NK cells for TCR gene therapy, aiming to overcome mispairing problem and enable specific intracellular antigen targeting by NK cells.

Method: NK92 and YTS cell lines were used in this study. Lentiviral transduction was performed to express the TCR complex in these cells. In vivo activity of TCR-expressing NK cells was tested by introducing fluorescence-labeled tumor cells into NOD/SCID mice. Tumors were injected subcutaneously into the right and left sides of the mice. NK-TCR cells were also given to mice on determined days. Measurements were taken regularly.

Results: TCR-NK cells efficiently detect an antigenic epitope presented by MHC-I and robustly kill targets expressing the antigen. TCR modification equips NK cells with the capacity to show antigen-specific cytotoxic activity both in-vitro and in-vivo while circumventing the potential risk of TCR mispairing. TCR expressing NK-92 cells can efficiently target tyrosinase expressing tumor cells using a NOD/SCID animal model and restrict tumor growth.

Discussion: This study underlines the value of NK cells as a resource that has similar cytotoxic capacity with T cells but present themselves unburdened from endogenous TCR expression. Expressing functional TCRs in NK cells stands out as a unique discovery combining robust and effective cytotoxic capacity of NK cells with the exclusive antigen specificity of T cells.
PURIFICATION AND CHARACTERIZATION OF MELANOMA ANTIGENS (MELANA OR TRP1)-SPECIFIC B CELLS IN MELANOMA PATIENTS

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Background And Aim B cells contribute to immune responses through antigen presentation and lymphoid tissue organization and antibody production. They represent the humoral arm of adaptive immune responses and can directly affect other cell types through their secreted cytokines. Melanoma is the most dangerous form of skin cancer and associated with a high morbidity and mortality. Although substantial progress has been made in our understanding of cutaneous immunity and skin tumors, particularly melanomas, the role of B cells still remains poorly defined. Checkpoint inhibitor (CI) therapies show great promise for the treatment several types of cancer. PD1- and CTLA4-targeting biologics have been approved for treatment of malignant melanoma. Most mechanistic studies in the field of CI therapy for cancer treatment has focused on the role of T cell responses to tumor antigens while the role of B cells and antibodies in this context remains largely unexplored. In this study we aim to characterize melanoma antigen-specific B cell responses in melanoma patients treated with CI inhibitor therapy. The data presented here are focused on establishing a method for detection and purification of B cells specific for melanoma antigens.

Methods: We used the recombinant melanoma antigens human tyrosinase-related protein 1 (TRP1) and MelanA (both contained a GST-tag) to set up a method for detection of melanoma-antigen-specific B cells. First, ELISA and western blots were performed to confirm the interaction between the antigens and monoclonal specific antibodies as well as GST-specific antibodies. Subsequently, in order to setup a flow-cytometry-based detection method, beads were coated with MelanA or TRP1-specific mAbs. These coated beads, which mimic antigen-specific B cells, were then incubated with different concentrations of recombinant GST-tagged MelanA or TRP1 followed by anti-GST-biotin and fluorescently labeled streptavidin. Using this optimized staining method, Melan-A and TRP1 antigen-specific B cells were labeled and purified from melanoma patient PBMC using FACS sorting. Purified melanoma antigen-specific B cells were immortalized through retroviral transduction with a retroviral vector including GFP, BCL6 and BCL-XL, and expanded in vitro with IL-21 and L-cells. Results Before the immortalization sorted cell numbers of Melan-A specific B cells were 43 besides TRP1 antigen-specific B cells count were 29 with the frequency 0.55% and 1.2% respectively. Although the numbers are low for antigen-specific B cells, after immortalization they are consistently growing in cell culture.
Following weeks, we will resort the cells to check the purity and specificity against their specific antigens. Before collecting cells from culture for flow cytometry, culture supernatant will be taken in order to capture the specific antibodies against their specific antigens with ELISA. This will be our chance to observe that how much amount of total IgG and antigen-specific IgG they produce These B cells will be further characterized by surface flow cytometry, secreted cytokines and RNAseq.

**Conclusion:** These preliminary data demonstrated that it is possible to Isolate, immortalize and characterize melanoma antigen-specific B cells and their role during CI treatment.
INVESTIGATING THE ROLE OF TRANSCRIPTION FACTORS FOR NKT10 CELL FUNCTIONALITY

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Background: iNKT cells recognize glycolipids presented by CD1d, an MHC class I-like molecule. NKT10 cells are a novel subset of iNKT cells that are characterized by IL-10 production. However, how IL-10 production is regulated in NKT10 cells, is not defined to date.

Purpose: The purpose of the study is to characterize the master transcription factor that drives NKT10 cell functions.

Methods: iNKT cells from control and antigen-challenged mice were FACS-sorted. The gene expression of iNKT cells was analysed by RT-PCR. Their phenotype and cytokine production were analysed by flow cytometry. Lentiviral vectors were prepared for the in vitro over-expression of the transcription factors Cebp-α, E4BP4, and c-Maf in iNKT cell lines.

Results: Our microarray data show that the three transcription factors known to bind to the IL-10 gene, Cebp-α, E4BP4, and c-Maf, are upregulated in NKT10 cells. Their expression was verified by RT-PCR. Lentiviral vectors for the transfection of all three transcription factors were cloned and verified by PCR and sequencing. Since the CMV promoter can be silenced in T cells due to antiviral defence mechanisms, the three vectors were also prepared using the PGK promoter. iNKT cell lines are currently expanded in vitro and will be used next for the lentiviral transduction.

Discussion: We expect that all the three transcription factors will be able to drive IL-10 production in iNKT cell lines when overexpressed. We hypothesize, however, that c-Maf is the master transcription factor that controls NKT10 cell functions under physiological conditions. The results from this study will greatly expand our knowledge of how NKT10 cells are regulated. Such understanding will facilitate subsequent research on human NKT10 cell in general and their therapeutic potential in particular.
The ATP-binding cassette transporter G1 (ABCG1) mediates efflux of cellular cholesterol to high-density lipoprotein (HDL) particles. Recent studies have shown that in mice in the absence of ABCG1, macrophages shift from a tumor-promoting M2 phenotype to a tumor-fighting M1 phenotype within the tumor and suppress bladder cancer growth in vivo. Confirming a similar tumour fighting potential of ABCG1 in human macrophages is the next crucial step to determine if ABCG1 can be a molecular target in cancer immunotherapy. To this end, we inhibited ABCG1 expression in human peripheral blood monocyte-derived macrophages by RNA interference before the macrophages were polarized to M1 or M2 phenotypes by the stimulation with LPS/IFNγ or IL-4, respectively. The expression of M1 and M2 markers were analysed by qPCR. The inhibition of ABCG1 by siRNA significantly increased the expression of the M1 markers IDO1, CXCL10, CD64 and TNF after LPS/IFNγ stimulation. In contrast, the inhibition of ABCG1 by siRNA significantly reduced the expression of the M2 markers MRC1, TGM2 and CD163 after IL-4 stimulation. Moreover, we showed that in human macrophages the expression of ABCG1 was negatively correlated with increased expression of M1 markers. Our results indicate that ABCG1-deficiency promotes macrophage polarization to an M1 phenotype in human monocyte-derived macrophages. These findings suggest that ABCG1 in macrophages could be a potential new target to modulate macrophage polarization for cancer immunotherapy.
INTRODUCTION: Acute Appendicitis (AA) is a condition characterized by inflammation of the appendix accompanied by systemic inflammatory response (1). Immune response and inflammatory factors are important in the complicated and uncomplicated appendicitis. Cytokines, white blood cell count (WBC), C-reactive protein (CRP), neutrophil-lymphocyte ratio (NLR) are important factors in determining the course of appendicitis. Concomitant evaluation of clinical and laboratory findings has a higher diagnostic value in cases with suspect diagnosis. The diagnostic value of interleukin (IL) 6 which triggers acute phase response as release of neutrophils and CRP is more important in diseases characterized by excessive production of acute phase proteins. Timely diagnosis of acute appendicitis is crucial for rapid progression to perforation, which may result in high rates of complications including wound infection, intra-abdominal abscess formation, and mortality. A delay in diagnosis of AA is associated with increased risk of perforation and further complications (1,2,3,4).

Aim: The purpose of this study was evaluation of IL1β, tumor necrosis factor-alpha (TNFα), IL6, CRP, WBC, NLR levels according to whether there were post op fistula, intra-abdominal abscess, wound infection and hospitalization time in uncomplicated, complicated groups.

Method: 120 patients who were suspected of AA (abdominal pain, vomiting etc.) and who were referred to the emergency-general surgery clinic and 30 healthy controls were enrolled in the study. Patients in whom the diagnosis of AA were documented with physical examination, radiologically and biochemically were included in the study and appendectomy was performed. Exclusion criteria comprised patients with a history of autoimmune disease, pregnancy; who underwent transplantation; receiving immunosuppressive/modulator, antibiotic drugs or who were <18 and >70 age. Ethical approval was taken from local committee of the Bakirkoy Dr Sadi Konuk Training and Research Hospital (2018/171). Patients were divided two groups as noncomplicated (acute or phlegmonous) appendicitis (n=81) and complicated (gangrenous or perforated) appendicitis (n=39). Serum levels of IL1β, TNFα and IL6 (pg/ml) were quantified by using enzyme-linked immunosorbent assay kits using Triturus analyzer (Grifols International, Spain), according to the manufacturers' instructions (Diaclone, France). The normal CRP (0.01-0.5 mg/dL) levels (immunoturbidimetrically), WBC (4-11x10³/uL) counts were implicated in the reference values used in our laboratories. Mann- Whitney-U, Kruskall Wallis H, Benfordoni tests and Spearman correlation analysis were used. A p value <0.05 was considered as statistically significant. NCSS 11 (Number Cruncher Statistical System) 2017 (Kaysville, Utah, USA) program was used for statistical analysis.

Findings: Median levels of all parameters in the complicated group were higher than the other groups (p<0.001). IL1β, TNFα, CRP levels of the who had post-op fistula, intraabdominal abscess, wound infection and also hospitalized patients were higher than the other groups (p<0.05). There was positive correlation between levels of parameters and hospitalization time (p<0.01). Discussion: Our study indicates that elevated levels of cytokines can be predictive and useful in the detection of clinical severity and follow up of the appendicitis following appendectomy in accordance with the other inflammatory parameters such as CRP and NLR. Increases in the levels, especially on the post op surgery, is crucial for detection of potential postoperative complications with clinical reflections.
FUNCTIONAL INTERACTION BETWEEN HELICOBACTER-STIMULATED B (HSTIM-B) CELLS AND DENDRITIC CELLS

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Introduction And Aim: Helicobacter pylori (H. pylori) is a microaerophilic gram negative and spiral-shaped bacteria that colonizes the gastric mucosa. More than half of the people in the world are infected with this bacteria; however, 10-20% of the infected people develop serious gastrointestinal diseases. It has been shown that Helicobacter felis (H. felis) stimulated regulatory B cells suppressed immune responses in vivo and in vitro by triggering IL-10-producing Tr1-like cells. This suppression prevents Helicobacter-mediated gastric pathology and contribute to persistency of bacteria within the gastric mucosa. Regulatory B cells produce anti-inflammatory cytokines such as IL-10 and TGF-β and suppress effector functions of dendritic cells. H. felis sonicate induces 20-30 % of B cells to produce IL-10. We aimed to determine the direct effects of H. felis sonicate and potential regulatory effects of Helicobacter felis-stimulated B (Hfstim-B) cells on immature bone marrow-derived dendritic cells (BM-DCs).

Method: C57BL/6 mouse bone marrow cells were differentiated into immature DCs using IL-4 and GM-CSF. Splenic B cells were magnetically isolated and treated with Helicobacter felis sonicate to obtain Hfstim-IL-10+ B and Hfstim-IL-10- B cells. Then, these B cell subgroups were stimulated with H. felis sonicate for 8 hours to obtain their supernatant. Immature BM-DCs were stimulated with supernatant of IL-10- B or IL-10+ B cells or co-cultured with total Hfstim-B cells for 24h at a ratio of 1:2 (DC:B) followed by LPS treatment for 12h. CD86 expression and cytokine profile of DCs were determined by flow cytometry and ELISA/qPCR; respectively.

Findings: H. felis antigens induced DCs to express CD86 and produce IL-1β, IL-12, TNF-alpha and IL-10. Supernatant of Hfstim-IL-10+ B or Hfstim-IL-10- B cells induced DCs to express CD86 and secrete IL-12. LPS treatment of DCs, which had previously been stimulated with the supernatant from IL-10- B or IL-10+ B cells, resulted in significant decrease in TNF-alpha secretion by DCs. Strikingly, supernatant of IL-10- B cells, but not IL-10+ B cells, led to a significant decrease in IL-10 secretion by DCs compared to control groups. In addition, our preliminary studies showed that cell-to-cell contact between total Hfstim-B cells and immature DCs followed by LPS treatment, induced DCs to produce significantly more IL-10 and less TNF-alpha compared to control group. Moreover, direct contact between total Hfstim-B cells and immature DCs activates DCs by upregulating CD86 expression.

Conclusion: Our study concluded that DCs were activated and secreted both pro-inflammatory and anti-inflammatory cytokines in response to H. felis antigens in vitro. Also, cytokines secreted by Hfstim-IL-10+ B cells together with cell-to-cell contact regulate the activation and function of DCs and convert immature DCs into IL-10-secreting tolerogenic form.
THE EFFECTS OF CTPS1 DEFICIENCY ON INNATE AND ADAPTIVE IMMUNE SYSTEM

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CTP-synthase-1 (CTPS1) is one of the two enzymes involved in cytidine triphosphate (CTP) production and its deficiency has been known as newly discovered immunodeficiency. The importance of this deficiency on the innate immune system is still unknown, although the significance of proliferation in lymphocyte cells has been demonstrated.

Here, we examined the innate and adaptive immune responses of a patient diagnosed with CTPS1 deficiency. Investigation of the whole blood lymphocytes revealed that CD4/CD8 ratio of the patient was found to be significantly different from healthy subjects. Moreover, a CD3+/CD8dim population was determined in the patient peripheral blood. When STAT3, STAT5 and STAT6 phosphorylation levels of CD4+ T-cells following stimulation with IL-6, IL-4 and IL-2, respectively, were examined by flow cytometry, it was determined that phosphorylation levels of these three STAT proteins were at least 2-fold lower than healthy subjects. The patient's PBMCs were found to contain CD14-CD15+ low-density granulocyte (LDG) population.

The amounts of IFN-γ, IL-4, IL-17a and IL-10 produced by PMA/Ionomycin stimulated cells were determined by intracellular cytokine staining. T-cells of the patient were found to secrete a lower amount of IFN-γ, IL-17a and IL-10 than T-cells of healthy controls. Isolated PBMCs were stimulated with TLR (Pam3CSK4, p (l: C), LPS, R848, D35 and K3 CpG ODN) and cytosolic sensor ligands (Nigericin, cGAMP, HSV, ATP) and cytokine release levels of the cells were determined by ELISA. The patient PBMCs stimulated with TLR4, TLR7, TLR9 (LPS, R848, K-ODN) ligands and cytosolic ligands (cGAMP, HSV-transfected) were found to produce low amounts of IFN-γ compared to healthy subjects. The patient PBMCs stimulated with TLR7 ligand were found to secrete high levels of IL-10 relative to healthy controls. The neutrophils isolated from the patient displayed an increased spontaneous NETotic activity (i.e. >4-5 fold more NETosis) compared to resting healthy neutrophils.

In conclusion, i) presence of higher LDG numbers along with ii) increased spontaneous NETotic activity of patient PBMCs might contribute to existing exacerbated inflammation. Additionally, iii) decreased phospho-STAT3/5/6 levels and iv) reduced IFN-γ levels might contribute to worsened immunodeficiency syndrome in CTPS1 patient.

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CHARACTERIZATION OF B & T CELLS IN NON-SMALL CELL LUNG CANCER

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Introduction And Aim: Lung cancer (LC) is the leading cause of cancer deaths worldwide. Tumor microenvironment mainly affects immunological responses via either suppressing effector cells or converting them to the regulatory cell types. Secretion of Interleukin-10 (IL-10) by regulatory cells contributes to suppression of the immune response in cancer microenvironment. This study aims to characterize immune cells in non-small cell lung cancer (NSCLC) according to their cell surface markers and cytokine profiles. It also aims to investigate the effect of tumor microenvironment on healthy B and T cells. Overall, this study especially aims to identify types of peripheral and tumor infiltrating regulatory B cells (Breg) that are not identified before in LC. Therefore, T cell (CD4, CD8), Breg (CD19, CD24, CD38) and PD-1 surface markers were examined along with anti-inflammatory (IL-10) and pro-inflammatory (IFNγ) cytokine profiles.

Method: We performed flow cytometry analysis of peripheral blood and tumor tissue samples collected from patients with NSCLC from Istanbul University, Cerrahpaşa Medical School, Thoracic Surgery Department. Peripheral Mononuclear Blood Cells (PBMC) were isolated from both healthy and patient samples. Tumor infiltrating lymphocytes (TIL) were obtained from tumor tissues that were resected during surgery. PBMC and TILs were stimulated with CpG ODN K3 (0.7μg/ml) for 24 hours and PMA/ionomycin for 5 hours, then stained with anti-CD4, anti-CD8, anti-CD19, anti-CD24, anti-CD38, anti-PD1, anti-IL10 and anti-IFNγ. To study the effects of NSCLC microenvironment, B cells and CD4+ T cells were purified from healthy PBMCs. Then, B and CD4+ T cells were cultured in either tumor or normal tissue conditioned medium which were obtained by culturing cells of tumor and normal tissue.

Findings: 7 healthy controls and 16 NSCLC patients were analyzed. IL-10 expression of CD4+ T and CD19+ B cells were increased in bloods (0.82-24.1%; 0.85-17.3%) and also in TILs (0-23.7%; 0-20%) of patients with NSCLC compared with healthy individuals (0-2.77%; 0.1-46%). CD8+IFNγ+ T cells were significantly decreased in TILs of LC patients to the healthy control level compared with their blood counterparts (20.3-94.9% to 3.0-53.2%). In addition, IFNγ producing CD4+ T cell frequencies were significantly decreased in bloods of LC patients (10.4-38.3% to 1.82-21.4%). IL-10 expression by CD19+CD24+CD38+ B cells (0-75%) from LC patients’ blood were greater than CD19+CD24+CD38+ B cells of healthy controls. PD-1 and IL-10 double positive B cells were significantly increased in PBMCs of LC patients (0.68-5.45%) in comparison to healthy individuals (0-0.94%). Moreover, our preliminary studies showed that IFNγ expression by CD4+ T cells decreased (15.2 to 10.7%) in tumor microenvironment compared to normal one. Also, IL-10 production was significantly elevated (0.34-1.26% to 9.21-6.51%) in healthy control B cells when they were cultured with supernatant of primary NSCLC tumor cells.

Conclusion: Our study concluded that the increased frequency of IL-10 producing B and T cells, PD1+IL-10+ Bregs and IL-10 producing peripheral CD19+CD24+CD38+ Bregs indicate an anti-inflammatory environment in NSCLC. In addition, decreased numbers of IFNγ producing CD8+ and CD4+ T cells suggest that B and T cell response shift to anti-inflammatory systemic and local responses.
CHARACTERIZATION OF CHRONIC SPINAL CORD INJURED URINARY AND PLASMA DERIVED EXOSOMES AND EXAMINATION OF THEIR NETOTIC TENDENCIES ON PATIENT AND HEALTHY NEUTROPHILS

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Spinal cord injury (SCI) is the damage on the spinal cord and the nerves which are found in the spinal canal and it causes loss of sensation below the injury site permanently. It is important to understand the role of immune response in SCI patients because uncontrolled immune response occurs on injury site following the trauma and this may increase the damage that lesion causes. According to the researches, inflammation in the injured site causes immune impairments such as presence of injury-dependent pro-inflammatory cytokines and infiltration of immune cells but the mechanisms that cause these responses needs to be further investigated. The aim of our study is to understand the underlying mechanisms of immune responses in SCI which may be the reason why patients are more prone to infections/inflammations and the responses of the patients to treatments are low. 2 veteran patients’ and 2 healthy controls’ neutrophils (2.5x10^5) were incubated with media or PMA (50ng/ml), also with plasma and exosomes (5μg) to determine amount of ROS production. It was observed that patients’ neutrophils had more respiratory burst at basal level but low level of ROS production upon PMA stimulation compared to healthy individuals. Then, neutrophils (4x10^5) of the same individuals were incubated with media or PMA (50ng/ml) for 4 hours and examined for extracellular DNA with EVOS. Spontaneous NETosis was determined for Patient 2 when the neutrophils were not stimulated but when stimulated with PMA, almost no difference in NETosis between patient and healthy neutrophils was observed. After that, neutrophils (2.5x10^5/well) were incubated with media or PMA (50ng/ml) again, also with plasma and exosomes (5μg) and after 4 hours, amount of released DNA was quantified with MNase. Strikingly, when the healthy and patient neutrophils were stimulated with patients’ exosomes, there were more released DNA compared to stimulated neutrophils with healthy exosomes. Finally, origin of healthy and patient exosomes were sought. Exosomes isolated from blood and urine were stained with Cell Trace Violet, a-CD63-PE, a-CD41-APC and a-CD42a-FITC in one panel. In another panel, these exosomes were first captured onto latex beads then coated with CD63 antibody and they were stained with a-CD81, a-CD3, a-CD19 and a-CD42a. These two panels were examined with NovoCyte flow cytometer. If exosomes were isolated from urine samples, data revealed that less patient exosomes were originated from platelets. Strikingly, blood plasma derived exosomes were mostly originated from T-cells and platelets compared to the exosomes derived from urine. Our investigation implied that neutrophils of SCI patients have more tendency to yield spontaneous NETosis. Furthermore, patient exosomes increased NETotic tendencies of healthy neutrophils and exosomes of patients and healthy individuals may be originated from different cells thus might display different pathological identities.
EVALUATION OF CLINICAL AND IMMUNOLOGICAL PARAMETERS OF PATIENTS WITH TACI MUTATION

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Transmembrane activator and calcium-modulator and cyclophilin ligand interactor molecule (TACI), a member of the TNF receptor family, is a molecule that is effective in APRIL/BAFF mediated terminal B lymphocyte differentiation and survival. It is stated that TACI deficiency is detected in 5-10% of patients with Common Variable Immunodeficiency (CVID) and a secondary mutation is thought to be necessary in the pathogenesis of the disease. The aim of this study is to investigate the clinical presentation and immunologic findings of 7 patients with TACI mutation detected by next generation sequencing targeted primary immunodeficiency gene panel in Department Of Pediatric Immunology, Hacettepe University Faculty Of Medicine, İhsan Doğramaci Children's Hospital. Six of the patients were diagnosed with CVID and a patient presented with selective IgA phenotype. TACI mutation was detected in 6.2% of the patients in our CVID cohort (n=65). The median age of the patients was 30 years. In addition to susceptibility to infections, 4 patients have autoimmune disease, a patient has ankylosing spondylitis, a patient has Guillain-Barré Syndrome and a patient has Chronic Granulomatous Disease (CGD). In the analysis of T lymphocyte subgroups of the patients, it was found that the number of naïve T cells were lower than the healthy controls and the number of effector memory cells were higher than the healthy controls. In the analysis of B lymphocyte subgroups of the patients, it was found that the number of naïve B cells were higher than healthy controls and the number of switched memory B cells was lower than the healthy controls. All patients have immunoglobulin (Ig) abnormalities. Homozygous TACI (C104R) mutation was detected in 2 cases and heterozygous mutations were found in other patients. C.310T>C was found in 4 patients, c.226G>A mutation was found in 2 patients and c.260T>A mutation was found in one patient. All mutations are in the CRD1 domain which is responsible for binding to APRIL and BAFF. In this study, TACI mutations were thought to be an important factor in the predisposition to infections and pathogenesis of autoimmune diseases.
REPEATED ENCOUNTER WITH ACTIVATED PBMC MEDIATES THE PROGRESSION OF IMMUNE RESISTANCE IN THP-1 MYELOID LEUKEMIA CELLS

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**Introduction:** Solid and hematological malignancies share numerous immune escape pathways, however several unique pathways are exploited by leukemia and lymphoma, which advances and disseminate somewhat differently than solid tumors, the pathways that regulate immune activation or tolerance are less clear. The evolvement of leukemia in a compartment associated with the immune responses suggests that it might fail to prompt immune sensing mechanisms (immunological ignorance) or effectively deteriorate anti-tumor immune responses when they do occur (immune evasion). The aim of this study is to determine the effect of repeated hits by allogeneic peripheral blood mononuclear cells (PBMC) on the progression of immune resistance in THP-1 acute myeloid leukemia (AML) cells.

**Methods:** PBMC were labelled with CFSE and co-cultured with THP-1 cells in the presence of stimulatory anti-CD3 mAb. Co-cultures were performed for 48 hours with 1: 0.25 ratio. The cell viability was tested (propidium iodide staining) and viable THP-1 cells were purified out of the co-cultures (back-sorting) by FACS. Next, the back-sorted THP-1 and the naïve THP-1 cells were labelled with CFSE and used in the co-cultures again with fresh eFluor670-labelled PBMC at different ratios (1:0, 1:0.25, 1:0.5, 1:1) for 48 and 72 hours. In the co-cultures, HLA-DR, PD-L1, PD-L2, CD14 expression, proliferation rate, changes in CD4⁺ to CD8⁺ ratio and viability were assessed by flow cytometry.

**Results:** The back-sorted THP-1 that were previously survived the co-culturing with activated T cells, was not negatively influenced even by the highest amount of PBMC in terms of viability and proliferation; on the contrary, naïve THP-1 cells’ viability was decreased as the ratio of PBMC increased. Further, an increase in surface expression markers HLA-DR, PD-L1, PD-L2, and CD14 was observed for on back-sorted THP-1.

**Conclusion:** This study is an attempt to show that the resistivity in acute myeloid leukemia cell line can be invoked by giving repeated hits by co-culturing with PBMCs. These preliminary results emphasize that a state of hypo-responsiveness can be produced under continuous hits by PBMC in vitro.
THE INVESTIGATION OF CORRELATION BETWEEN KREC NUMBERS AND B CELL SUBGROUPS OF PATIENTS WITH COMMON VARIABLE IMMUNODEFICIENCY

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Common Variable Immunodeficiency (CVID) comprises a heterogeneous group diseases characterized by recurrent infections, poor response to vaccines, impaired B cell differentiation and antibody production. In recent years, attempts in classifying the disease based on phenotyping B-cell populations have been proposed for follow-up and treatment integrity. Kappa-deleting Recombination Excision Circles (KRECs) are episomal DNA segments that occur during V(D)J rearrangement of B Cell Receptor (BCR) genes in bone marrow. 30 patients diagnosed with CVID in Section of Pediatric Immunology, Hacettepe University, İhsan Doğramacı Children's Hospital were included in this study. B cell subgroups of CVID patients were evaluated by flow cytometry, KREC numbers of the patients were measured with RT-qPCR and evaluated in comparison with 41 healthy controls.

The mean age of the patients was 28.5±15.6 years. 33% of patients have consanguineous parents. The most common complaint of admission was frequent infection. CD19+ cell counts were low in 20 patients (66.67%) while 14 patients (46.67%) had low CD4+ cell numbers. There was a decrease in the number of B cells of the controls with the increasing age (p<0.01). Transitional B cells and naive B cells were inversely correlated with age (p<0.01). KREC levels was inversely correlated with age only in healthy controls (p<0.01). In the patient group, the level of KRECs (median:1644 copies/ml) was found to be lower than the control group (median:10477 copies/ml) (p <0.05). In the study, B cell subgroup numbers and KREC levels in the normal population were obtained according to age. Evaluation of the inverse correlation between B cells/KREC levels and age of controls may also be important in terms of demonstration of immune aging.
HYPOCRISY OF HODGKIN’S LYMPHOMA CELLS IN IMMUNOMODULATION OF T CELLS REGARDLESS TO COMPARABLE EXPRESSION OF B7 COSTIMULATORY MOLECULES

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Introduction and Aim: As it is generally located in secondary lymphoid tissues, Hodgkin’s lymphoma (HL) constantly encounters with immune cells, primarily T cells. Yet, current knowledge about the interactions between HL cells and T cells are limited. B and T cell communication is critical for activation of both parties where the costimulation axis sustained by B cells are responsible for initiation and modulation of T cell responses. Previously, our group showed adaptive resistance mechanisms of AML cells through B7 costimulatory ligands leading to functional exhaustion of T cells. Thus, the aim of this project is to determine the co-stimulatory capacity of HL cells and their effect on initiation and regulation of T cell activation or exhaustion (functional hypo-responsiveness).

Methods: Two Hodgkin lymphoma cell lines (HDLM-2 and L428) were cultured under standard conditions. Expression of T cell interaction/costimulatory molecules; HLA-DP, HLA-DQ, HLA-DR, B7-H1, B7-H2, B7-H3, B7-H4, B7-DC, CD2, CD19, CD20, CD38, CD40, CD58, CD62L, CD69, CD70, CD80, CD86 molecules were assessed with flow cytometry. HL cells were co-cultured at different ratios with lymphocytes. T cell responses were assayed with flow cytometry; CD69, CD40L, CD25 expression were determined for activation, eFluor670 dilution assay was used for proliferation and PD-1, TIM-3, LAG3 levels were examined for exhaustion. The presence of these co-inhibitory TIM-3 and LAG3 receptors on CD3+ cells was tested with immunohistochemistry (IHC) on HL patient samples.

Findings: Both HL cell lines highly expressed HLA-DQ, HLA-DR, B7-H2, CD70, CD80 and CD86 and to a lesser extent CD40. Inhibitory B7-DC and B7-H3 molecules were also present on L428 cell line. Co-culture of L428 HL cells with CD4+ or CD8+ T cells significantly elevated CD69 and CD25 activation markers and augmented T cell proliferation which indicated efficient co-stimulatory signals provided by L428. Importantly, exhaustion-related PD-1, TIM-3 and LAG3 expression were also detected on CD4+ T cells after long-term co-culturing. However, neither activation/proliferation nor exhaustion markers were significantly observed on T cells when co-cultured with HDLM-2 HL cells. Only ~50% of the patient-derived HL samples, harbored T cells that expressed LAG3 which not always accompanied with TIM-3.

Conclusion: Our findings may contribute to understanding immune regulatory capacities of HL cells. Even though both cell lines expressed similar levels of B7 family costimulatory molecules L428 and HDLM-2 may represent two distinct HL cell regards to their functional impact on T cell responses. These findings may demonstrate the heterogeneity of immune modulatory facets in HL.

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AN INFLAMMATORY POSITIVE FEEDBACK MECHANISM BETWEEN FIBRONECTIN AND IL-1B IN BASAL-LIKE BREAST CANCER THROUGH MONOCYTE

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Introduction and Aim: Components of extracellular matrix such fibronectin, can support myeloid cells differentiation and maturation. Fibronectin EDA (FN-EDA) is the uppermost FN isoform with immune-associated functions mediated through TLR4 and integrin molecules. This study aims to reveal the inflammatory role of FN secreted from basal-like breast cancer (BLBC) cells.

Methods: BLBC cells (MDA-MB-468, MDA-MB-231, and HCC38) were stimulated with IL-1β. Total fibronectin and FN-EDA expression was evaluated by RT-PCR and Western-Blot, adn ELISA. Peripheral blood monocytes and THP-1 cell line were cultured with BLBC supernatants with or without recombinant derivatives of FN isoforms (plasma FN, FN-EDA-, FN-EDA+) and IL-1β production were determined by RT-PCR and ELISA. NF-κB activity was assessed by HEK-BlueTM Null2 cells with SEAP reporter were used.

Findings: Total fibronectin and FN-EDA isoform were abundant in BLBC (MDA-MB-231, HCC38) and increased upon exposure to IL-1β. Moreover, BLBC supernatants augmented IL-1β production from monocytes and THP-1 cells. Furthermore, when FN-EDA levels were exogenously increased in the BLBC supernatants, maximal levels of IL-1β was secreted by the monocytes. The influence of plasma FN and FN-EDA- was not as strong as that of FN-EDA+ isoform. Neither the BLBC supernatants nor the recombinant FN isoforms alone could directly induce NF-κB activity in the reporter system. Then, monocytes were stimulated BLBC supernatants and FN isoforms and supernatants were collected from these monocytes. Accordingly, high signals from NF-κB activity was recorded with the secreted factors from monocytes that were incubated with BLBC supernatants and FN-EDA+ isoform.

Conclusion: A positive feedback loop exist in basal-like breast cancer cells and monocytes through IL-1β and FN-EDA isoform which may support chronicity of inflammation in the tumor microenvironment.

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EXTRACELLULAR VESICLES ISOLATED FROM PATIENTS UNDERGOING GRASS POLLEN IMMUNOTHERAPY INDUCE ALLERGEN-SPECIFIC TH17 AND ANTI-ALLERGEN RESPONSES

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Allergen immunotherapy (AIT) is the most effective method to achieve long-term remission and cure for allergic diseases. How long-term AIT induces allergen-specific tolerance is not entirely understood. Conflicting reports exist concerning the contribution of Th17 cells some of which indicating a protective role in AIT. Also, how circulating extracellular vesicles (EVs) are altered by AIT and if they contribute to the therapeutic outcome are unresolved issues which this study aims to challenge.

17 patients receiving grass pollen AIT were monitored for 3 years. PBMCs were isolated before and each year during AIT. EVs from patient plasmas were isolated using differential ultracentrifugation and characterized by bead-based flow cytometry assays, immunoblotting and atomic force microscopy. Changes in T-cell population percent were determined with flow cytometry in basal state, after PMA-ionomycin and in-vitro allergen stimulations. To investigate whether EVs contribute to cellular changes associated with AIT, healthy and patient PBMCs were stimulated with pre- and post-therapy EVs of patients and with healthy EVs as control. Cytokine levels from culture supernatants were determined with ELISA.

In accord with the literature, Th1 and Treg responses increased upon AIT. Surprisingly, Th17 cell population and IL-17 production were significantly elevated after 1 year of AIT and stayed elevated for up to 3 years period. Furthermore, this Th17 response was antigen- specific. Post-therapy EVs of 2nd and 3rd year induced significantly higher IFNγ, IL-10 and IL-17 production from patients’ own PBMCs and from healthy PBMCs compared to pre-therapy EVs.

These results indicate that development of a tolerogenic allergen-specific Th17 response was induced by long-term AIT. The study also demonstrates for the first time that EV profiles were rehabilitated by AIT and EVs might be contributing to the establishment of an allergen-specific tolerogenic cellular changes governed by AIT.

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EXOSOMES CO-ENCAPSULATING ANTIGEN AND IMMUNOADJUVANTS ACT AS AN EFFECTIVE THERAPEUTIC CANCER VACCINE

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Exosomes are natural nano-vesicles (~30-150nm) secreted from many cells. Recent studies suggested that exosomes are excellent vehicles for protein/peptide, gene or short sequences of RNA/DNA delivery. Although dendritic cell derived exosomes are used in the immunotherapy of cancers, multiple ligand loading is not possible via cell feeding approach. Herein, we describe a simple method to externally load ligands within cell-line derived exosomes.

Exosomes were first purified from the cell supernatant via gradient ultracentrifugation method and loaded with i) cancer antigen ovalbumin (OVA), ii) CpG ODN (an immunoadjuvant), and iii) α-GalCer an iNKT-specific lipidic adjuvant. Therapeutic effect of these exosomes was tested on B16 F10-OVA model. After palpable tumor formation, animals were treated twice either with mixture of 5 µg OVA, 2 µg ODN and 0.06 µg αGC or their co-loaded forms within 30 µg of exosomes. Tumor regression was followed daily by an electronic caliper measurement and reported as volume (mm³). At the end of the experiment tumor infiltrating lymphocytes (TIL) were analyzed by flow cytometry from tumor cell suspensions. Therapeutic effect of the exosome formulation was evaluated by OVA-specific ELISA from the mouse sera (at day:10, post-therapy). Also, splenocytes were treated with MHC-I peptide specific epitope for 72h and CD8+ T-cell specific IFNγ secretion was detected from cell supernatants via ELISA.

We found that triple ligand loaded exosome injected group showed significant regression in tumor volumes compared to PBS or free mixture treated groups (330±120, 1180±440mm³, 1850±250mm³ exosome vaccine loaded with ligands, free ligand mixture vaccine and PBS treated group, respectively). OVA-specific antibody titers showed a Th1-biased anti-OVA immune response after a single injection of exosome formulation (IgG2c/IgG1>1.15±0.3 vs 0.56±0.2; exosome loaded ligand vs free ligand mixture group). Flow cytometry analyses revealed that tumor infiltrating T-cells, CD8+ T-cells, CD4+ T-cells, NK-cells, NKT-cells, pan- and M1-like macrophages were significantly high in exosome vaccine treated animals compared to PBS or free ligand mixture vaccine treated groups. Neutrophilic MDSCs and M2-like macrophages were not different in exosome received animals compared to PBS or free mixture vaccine treated groups. Also, we found that when MHC-I peptide epitope was incubated with splenocytes, CD8+ T-cells had significantly higher levels of IFNγ secretion compared to PBS or free mixture vaccine treated groups (i.e. 493±120pg/ml vs. 16±4pg/ml; exosome vaccine vs. antigen+adjuvant+αGC group).

In conclusion, this study suggested that exosomes could be externally loaded with multiple ligands via lyophilization method and these exosomes provide sufficient immune activation and robust antigen-specific immunity capable of reducing established melanoma in mice.

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INVESTIGATION OF T CELL RECEPTOR VB REPERTOIRE IN PATIENTS WITH TACI DEFECTS

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TCR (T Cell Receptor) are receptors responsible for recognizing antigens presented by HLA molecules of the APC (Antigen Presenting Cells) expressed on T cells and initiating an inflammatory response. ~90% of T cells consist of αβ T cells. In αβ T cells, TCR is a heterodimer of α and β chains and contains variable regions on both α and β chain. These variable regions differ by ~10 15 - 10 20 with some factors such as HLA diversity and TCR gene polymorphisms and external factors such as viruses, superantigens. It has been reported that TACI (Transmembrane activator and calcium modulating cyclophilin ligand interactor) mediated interaction of B cells and dendritic cells which are responsible for the processing of T cells. TACI is an important molecule in the development and B cell survival. In TACI deficiency, common variable immunodeficiency were seen characterized by low levels of IgG, IgM, IgA. B cell maturation, impaired somatic hypermutation and decreased memory, and decreased memory B cell count. In this study, 7 patients with TACI gene defect [2 homozygous (c.310T> C), 5 heterozygote (c.310T> C, c.226G> C, c.260T> A)], TCRVB clones were analyzed by using multiparametric flow cytometry method. A statistically significant decrease was determined in TCRVB8 clone in 24 TCRVB clones compared to healthy controls in patients with TACI defect (p<0.05) and an increase was detected in TCRVB13.2 clone.
EFFECT OF THE MESENCHYMAL STROMAL CELLS STIMULATED WITH CAFFEIC ACID PHENETHYL ESTER (CAPE) ON DIFFERENT CANCER CELL LINES

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Introduction: In cancer treatment, in addition to traditional therapies such as gene therapy, chemotherapy and radiotherapy, various alternative therapies are being developed. In recent years, various natural and biologically active compounds have begun to become popular research topics as an alternative therapy. Mesenchymal stromal cells (MSCs) are found in many tissues, and have the ability to differentiate into cells and tissues. They also play an important role in the regulation of the secretory cells and the cells in the environment. Cancer cells have similar properties and receptors to mesenchymal stromal cells and interact with each other in the niche. Mesenchymal stromal cell subsets show different effects. While MSC 1 stop the growth of cancer cells, MSC 2 stimulate the growth, development and spread of the cancer cells.

Methods: In our study, we have used the CAPE (caffeic acid phenethyl ester) which is one of the active ingredients of propolis, and is known to have antioxidant, anti-microbial, anti-inflammatory, anti-hypertensive and anti-cancer effects. The aim of this project is to determine therapeutic effect of CAPE stimulated mesenchymal stromal cells on the A172 (glioblastoma) and A549 (human lung carcinoma) cell lines. Serially diluted doses of CAPE (10μM, 25μM, 50μM, 75μM, 100μM), were applied on to mesenchymal stromal cells. Results Cell viability was determined by MTS method and the most harmless dose was found to be 10 μM. This dose was added to the mesenchymal stromal cells and incubated. Then cross-medium exchange was applied between MSC and cancer cell lines for 24 to 48 hours. The total cell death of the A172 cell line was approximately 20-30% in time, but cross-medium exchange did not have much effect on A549 cell line. The death rates in three groups of cross medium exchanged samples (Control, MSC + A172 medium, A549 + MSC medium) were 4% and similar.

Conclusion: Our preliminary results have shown that CAPE is a potential anti-cancer agent that can be used in combination with mesenchymal stromal cells in cancer treatment to potentiate its effect.
IDENTIFICATION OF CELL-TYPES RESPONSIBLE FOR MYD88-DEPENDENT MALARIA-INDUCED BONE LOSS

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Introduction: Malaria infection causes ~250 million acute illnesses, half a million deaths and many complications each year (1). Chronic bone loss during malaria infection is one of these complications and it is mediated by over-activation of osteoclast resorption activity. The osteoclasts are activated by the key osteoclastogenic cytokine RANKL, which is upregulated in osteoblasts through MyD88 dependent signalling triggered by the persistence of parasite products in the bone marrow (2). Purpose: Our previous results have suggested that Rag2−/− mice, which lack of T and B cells, had severe bone loss similar to their WT controls suggesting minimal role of adaptive immune system on malaria-induced bone loss. Based on this evidence, we aimed to investigate the precise contribution of specific cell type(s) belonging to innate immune system for controlling inflammation and bone loss during Plasmodium infection.

Method: To achieve our goal, we generated cell type specific MyD88 deficient mice based on Lox-Cre system. Mice with same age and gender were infected with P. yoelii Non-Lethal strain. Femurs were collected for bone morphometric analysis by micro CT after recovery from malaria infection. Results: We have evaluated the bones of mice lack of MyD88 gene in macrophages/osteoclasts (MyD88flox/flox LySMcre), in osteoblasts (MyD88flox/flox Col1acre), and in DCs (MyD88flox/flox CD11ccre) after malaria infection. We will discuss our results in this presentation.

CYTOKINE PROFILES OF THE PATIENT WITH A RARE DEFECT OF RLTPR

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Introduction: The RLTPR (RGD motif, leucine rich repeats, tropomodulin domain and proline-rich containing) cytosolic protein, also known as CARMIL2, is essential for co-stimulation via CD28, T cell differentiation, activation, and the development of regulatory T cells1. Furthermore, the cells with RLTPR defect show impaired cytoskeletal microtubule network and defective migratory pattern with increased spontaneous motility1.

Aim: The aim of this study was to investigate the cytokine profile in response to different innate sensor ligand stimulations of the patient PBMCs with RLTPR defect that causes primary immunodeficiency disease.

Methods: Peripheral blood mononuclear cells (PBMCs) of the patient and two healthy controls were obtained from peripheral blood following centrifugation on a lymphocyte separation medium. The PBMCs were then stimulated with membrane bound and endosomal associated TLRs; poly(I:C), LPS, flagellin, R848, resiquimod, K-ODN, and D-ODN, inflammasomes activation was monitored following transfection with poly(I:C), LPS, flagellin, Alum, p(dA:dT), and finally cGAS-STING and AIM2 pathway triggering were induced by cGAMP and HSV for 24 hours. Cytokines levels of IFN-γ, IL-12, IL-1β, IFN-α, TNF-α, and IL-10 were measured by ELISA reader at 405nm. The cytokine profiles of the patient and two healthy controls were compared. Results: Proinflammatory cytokines including IL-12, IFN-γ, IL-1β, and TNF-α increased after the stimulation of TLR4 and TLR5. Whereas IL-12 and TNF-α responses to the stimulants for endosomal TLR7 and TLR7/8 were similar to the healthy controls. Of note, IFN-γ, IL-1β, and IFN-α levels were higher in response to the endosomal TLR7 and TLR7/8 stimulation. Cytokines of IL-12, IFN-γ, IL-1β, IFN-α and TNF-α increased due to overactive cGAS-STING and AIM2 pathways in the patient compared to the healthy controls. RIG-I/MAVS and NLRP3 pathways were shown to be more active in the patient than healthy controls via IL-1β measurements. In addition, noncanonical inflammasome, NLRP3, and NLRC4 pathways were also determined as more active in the patient than the healthy group after the measurement of TNF-α and IL-10.

Discussion: RLTPR acts as a scaffold protein that bridges CD28 to CARMA1, and is essential for CD28 co-stimulation2. In this study, we showed increased proinflammatory cytokines including IFN-γ, IL-12, IL-1β, IFN-α, and TNF-α after the stimulation of membrane bound and endosomal TLRs, also our data suggested that the patient showed a tendency to develop a pronounced Th1- biased immune response due to an overactive cGAS-STING, AIM2 pathways, noncanonical inflammasomes, NLRP3 and NLRPC4 pathways in the patient. These results suggest likely gain-of-function mutation in the gene of RLTPR in our patient. References: [1] PMID: 23793062 [2] PMID: 27647348

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POTENTIAL VACCINE ADJUVANT EFFECT OF SAPONINS FROM CEPHALARIA SPP

Ayse Nalbantsoy, Nazli Boke Sarikahya, Suheyla Kirmizigül

Introduction and Aim: Saponins isolated from a large number of plants have a broad spectrum of biological and pharmacological activity using in vitro and in vivo bioassays. The recent investigations in biological activity studies of saponins have mostly focused on immunomodulatory. These studies findings exhibited that saponins can be used as immunomodulatory compounds and vaccine adjuvants. Since 1950’s, saponins have been used as vaccine adjuvants in veterinary vaccines. Considering the great potential of saponins as vaccine adjuvants, the presented study focused on immunomodulatory activities of saponins isolated from the aerial parts of Cephalaria species from Turkey.

Method: The isolated saponins were tested for their hemolytic and immunomodulatory activities through an in vitro cell based assay systems. Immunomodulatory activity was performed in stimulated whole human blood cells by PMA plus ionomycin. Hemolytic activity was assessed on human erythrocytes.

Findings and Conclusion: The thirteen saponins out of the nineteen tested increased IL-1β concentrations considerably indicating the regulation of innate immune responses, while six compounds changed IL-2 or IFN-γ levels slightly by ELISA. Almost all of the saponins showed noticeable hemolysis in human erythrocyte cells based on the sugar units. In this study, the results can be addressed potential adjuvant application of some saponins isolated from Cephalaria species due to its direct activity on innate immune response and indirect activity on adaptive immune response by inducing IL-1β secretion.
HLA-DBA1/B1 GENE POLYMORPHISMS RS3077 AND RS9277535 MAY INFLUENCE OUTCOME OF HEPATITIS B INFECTION AND BECOME A RISK FACTOR FOR CHRONIC HEPATITIS D

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Background and Aims: In Hepatitis B infection, human leucocyte antigen (HLA) class II gene polymorphisms influence immune host response by varying antigen presentation efficiency. Recent Genome Wide Association (GWA) studies have revealed that single nucleotid polymorhisms (SNPs) including rs3077 at the HLA-DPA1 and rs9277535 at the HLA-DPB1 are associated with Hepatitis B infection persistency as well as spontaneous recovery. Besides, due to dependency of HDV on HBV infection, we hypothesized that these SNPs can also be a risk factor for chronic HDV. Therefore, we aimed to assess association of HLA-DP gene polymorphisms with susceptibility to Chronic Hepatitis B and D infections in Turkish population.

Methods: The study was conducted with a total of 599 Turkish in three cohorts: First cohort was composed of total 261 patients with HBsAg persistence, including 48 inactive carrier patients, 145 HBeAg- positive Chronic Hepatitis B (CHB) patients and 68 Anti- HBe-positive CHB patients. Second Cohort was the control group including 101 participants having natural HBsAg seroclearance with Anti- HBs and Anti- HBc positivity. A total of 211 chronic hepatitis delta (CHD) patients with Anti-Delta and HDV-RNA positivity constituted the Third Cohort. The SNPs (rs3077 and rs9277535) were genotyped by using melting curve analysis using Real-Time Polymerase Chain Reaction (RT-PCR).

Results: A significant difference was observed for both rs3077 (p= 0.041) and rs9277535 (p<0.001) when HBsAg persistence was compared with spontaneous recovery. rs9277535 was also highly significant in comparisons of chronic HBV vs inactive HBsAg carriers (p<0.001), HBsAg persistence vs CHD (p<0.001) and HBeAg negative vs HBeAg positive (p<0.001), while no statistical significance was found between these groups for rs3077.

Conclusion: Our results revealed that risk allele (C) for rs3077 SNP and the risk allele (G) for rs9277535 SNP may be associated with HBsAg persistence in HBV patients. Moreover, the latter may also contribute to chronic HDV infections while interfering with the HBeAg seroconversion in HBV infected patients.
In this study, we aimed to determine the distribution of iNKT cell subsets in several murine organs under resting conditions. We initially optimized our protocols for the purification of iNKT cells. We showed that purification with a density gradient or blocking of P2X7Rs with an antagonist can improve iNKT cell recovery and functionality. Furthermore, we optimized the calcium concentration during in vitro stimulation to augment the cytokine production by iNKT cells. Using these methods, we determined the cell frequency and cytokine production of NKT1, NKT2, NKT10, and NKT17 cell subsets from various murine organs by flow cytometry. Knowing the resting state distribution of iNKT cell subsets is essential to monitor alterations that may occur in disease states. Changes in the subset ratios could be indicative of the role iNKT cells play in disease pathogenesis and may provide novel alternatives for therapies.
PERIPHERAL NKT2 CELLS APPEAR TO BE HYPORESPONSIVE IN MICE

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Invariant natural killer T (iNKT) cells are unique subsets of T cells that recognize glycolipids via CD1d, a non-polymorphic MHC class I like molecule. They bear an invariant TCRα chain comprised of Vα14 (mouse) or Vα24 (human) together with Jα18. Following activation, iNKT cells can rapidly secrete large amounts of cytokines. iNKT cells differentiate into functional distinct subsets that are characterized by their expression of particular transcription factors and cytokines. It is thought that NKT1, NKT2, NKT17, NKT10 and NKTFH cells mirror the conventional CD4+ T cell subsets of Th1, Th2, Th17, Treg and ThFH cells. Consequently, NKT2 cells are considered to be major source of iNKT cell-derived IL-4, based on data using IL-4 reporter mice. However, our data demonstrate that splenic NKT2 cells produced less IL-4 than NKT1 cells after activation with either αGalCer or PMA/Ionomycin. Similar, the expression of cytokines GM-CSF, IFNγ, IL-2, IL-4, IL-13, and TNF was lower in splenic NKT2 than in NKT1 cells. Therefore, we hypothesize that peripheral NKT2 cells are hyporesponsive. Additional work on cell signaling (Nur77, pERK1/2) and cytotoxic potential of NKT2 cells are ongoing to confirm this hypothesis. Knowledge of the main source of iNKT cell-derived Th2-cytokines is essential to understand the role iNKT cells can play in various diseases and to improve iNKT cell-related diagnosis and therapy.
THE ASSOCIATION BETWEEN INTERLEUKIN-21 GENE POLYMORPHISM AND INTERLEUKIN-21 SERUM LEVELS WITH BEHÇET’S DISEASE SUSCEPTIBILITY

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Background: Behçet's disease (BD) is a chronic auto inflammatory disorder of unknown etiology. Interleukin-21 (IL-21) is a newly discovered member of the Type-I cytokine family which has a variety of effects on the immune system. Previous studies have identified an association between IL-21 gene polymorphisms and different autoimmune and auto-inflammatory diseases, such as Systemic Lupus Erythematosus, rheumatoid arthritis and Type 1 diabetes. Herein, we aimed to investigate, for the first time, the association between IL-21 gene rs1314866 polymorphism and IL-21 serum levels with BD susceptibility and its clinical manifestations in a Tunisian cohort.

Methods: 65 BD patients and 116 age and sex matched healthy controls were genotyped for rs13143866 IL-21 gene polymorphism, using mutagenically separated PCR method. Serum IL-21 levels were measured by an enzyme-linked immunosorbent assay (ELISA) commercial kit.

Results: We found a significant difference in the genotype and allele frequencies distribution of IL-21 gene rs13143866 polymorphism between BD patients and the healthy control (p= 0.031). The rs13143866 T minor allele was found to be associated with BD susceptibility (OR = 1.86, 95% CI = 1.18-2.93, p = 0.006). The TT genotype was found to be associated with BD under the recessive model (OR = 2.65, 95% CI = 1.09-6.44, p = 0.027). Elsewhere, we found a significant association of serum IL-21 levels with BD activity (p=0.027). In addition, our results revealed a significant correlation of serum IL-21 levels with the presence of ocular involvement (P=0.025) and neurological manifestations (P=0.05).

Conclusion: Our study detected, for the first time, an association between IL-21 gene variant rs1314866 and BD. Serum IL-21 levels were correlated with BD activity and its clinical manifestations in our cohort.
ASSOCIATION BETWEEN AGER, ADAM10 AND FURIN POLYMORPHISMS IN THE DEVELOPMENT OF MICROANGIOPATHY IN PATIENTS WITH TYPE 1 DIABETES

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Background: Advanced glycation is one of the major pathways involved in the development and progression of different diabetic complications including nephropathy, retinopathy and neuropathy. Several candidate genes could be associated with diabetic microangiopathy. The aim of our study was therefore to explore whether polymorphisms (AGER rs1800625, AGER rs1800624, ADAM10 rs653765, ADAM10 rs514049, FURIN rs4932178 and FURIN rs6227) in these genes are associated with the presence of diabetic complications. Methodology: The studied polymorphisms were genotyped in 145 Tunisian adults with type 1 diabetes, recruited after a follow-up of 10 years minimum from diabetes onset, as well as in 187 non-diabetic control subjects.

Principal Findings: Our results indicate that the 6 polymorphisms of the genes we choose to study are strongly associated with neuropathy in Tunisian type-1 diabetic patients. The ADAM10 rs653765 C allele was detected in 34.5 % DT1 patients with nephropathy and 46.55 % DT1 patients without nephropathy thus the C allele confers some protection against the development of nephropathy (OR=0.6 , 95%CI: [0.37-0.98],p=0.04). On the other hand, the association between retinopathy and the 6 polymorphisms of the advanced glycation pathway did not reached significance.

Conclusions: Our results show an association between the studied polymorphisms and type 1 diabetes microangiopathy. All polymorphisms were associated with diabetic neuropathy and ADAM10 rs653765 was associated with nephropathy in the Tunisian population. This study for the first time ever reports the association of ADAM10 and FURIN with the development of microangiopathy in patients with type 1 diabetes.
IL-21 RECEPTOR RS2285452 POLYMORPHISM IS ASSOCIATED WITH BEHÇET'S DISEASE SUSCEPTIBILITY IN A TUNISIAN COHORT

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Background: Behçet's disease (BD) is a chronic auto inflammatory disorder of unknown etiology. The dysregulation of Interleukin-21 receptor (IL-21R) gene was, recently, associated with different autoimmune and auto-inflammatory diseases, such as Systemic Lupus Erythematosus, rheumatoid arthritis and Type 1 diabetes. Herein, we aimed to investigate for the first time ever the association between two polymorphisms in IL-21R gene and BD in a Tunisian cohort.

Methods: IL-21R gene polymorphisms rs2214537 and rs2285452 were explored in a cohort of 65 adult BD patients and 116 matched healthy controls. Genotyping was performed using mutagenically separated polymerase chain reaction (MS-PCR) with newly designed primers.

Results: IL-21R rs2285452 genotypes and alleles distributions were statistically different between BD patients and the control group. GA and AA genotypes carrying the minor A allele were more frequent in BD patients than in healthy controls (33.8% and 10.8% vs 23.3% and 3.4%, respectively, p=0.025). The association of IL-21R rs2285452 genotypes with BD susceptibility was more significant when the dominant model was used [GG vs GA+AA; p=0.014, OR=2.209, 95% CI=1.166-4.185]. The A allele was found to be associated with BD susceptibility (OR=2.155, 95% CI=1.273-3.648, p = 0.003). In contrast, IL-21R rs2214537 was found not to be associated with BD susceptibility in any genetic models (p>0.05). No association between the different clinical manifestations of BD and IL-21R gene two polymorphisms was found in our study. The IL-21R rs2285452 was associated with BD susceptibility in our study cohort. Functional studies are required to investigate its exact role in BD.
STUDY OF ASSOCIATION OF POLYMORPHISM RS2280964 OF CXCR3 GENE WITH TD1 IN TUNISIA

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Background: Type 1 diabetes (T1D) is a multifactorial disease that is due to progressive autoimmune β pancreatic islet cells destruction CXCL10, a Th1 chemokine with chemotactic activity, is induced by human pancreatic β cells following EV infection. CXCL10, acting through its receptor CXCR3, allows the migration of activated lymphocytes Th1 to the pancreatic inflammatory site, where they play an important role in the destruction of β cells. Some polymorphisms have been described for CXCR3 gene and associated with different autoimmune and infectious diseases. However, no studies have been done in T1D. OBJECTIVE: To investigate an association between polymorphism rs2280964 of CXCR3 gene, GADA and IA-2A autoimmunity, and T1D in Tunisia.

Material And Methods: This case-control study was conducted over a two-year period. A total of 315 patients with T1D and 411 healthy controls were investigated for CXCR3 intron 1 polymorphism rs2280964 by MS-PCR analysis. Statistical analysis was performed by the SPSS software.

Results: There were no significant differences in the genotype and allele frequencies distribution of CXCR3 gene rs2280964 polymorphism between patients with T1D and the control subjects. We also show no significant difference in the genotype frequency distribution of CXCR3 rs2280964 between GADA+ and GADA- diabetic subjects (p = 0.07) and between IA-2A+ and IA-2A- diabetic subjects (p = 0.9). Nephropathy, neurology and retinopathy were not associated with the rs2280964 polymorphism. CONCLUSIONS: The CXCR3 polymorphism rs2280964 was not associated with T1D in Tunisia.
EVALUATION OF CO-STIMULATORY MOLECULE EXPRESSIONS OF PMA AND LPS STIMULATED THP-1 CELLS

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Introduction and Aim: Monocyte and macrophage cells are phagocytic cells and are important components of innate immune response. However, another important role for these cells is the initiation and maintenance of the adaptive immune response by antigen presentation. Successful T cell activation requires multiple signals. Signal 1 is generated by presentation of an antigen bind to cell surface major histocompatibility complex (MHC) molecules on antigen-presenting cells to a specific T cell receptor (TCR) whereas signal 2 is generated via an interaction between members of the B7 family (either CD80 or CD86) on antigen-presenting cells and CD28 on T cells. In the absence of signal 2, T cells become anergic. In the absence of both 2 signals, T cells become unresponsive. THP-1 cells are a cell line that has been isolated from the acute monistic leukemia patient. In contrast to normal monocyte cells, THP-1 cells can proliferate in vitro and differentiate into functional macrophage cells by Phorbol 12-myristate 13-acetate (PMA) stimulation. Therefore, it is widely used to investigate the immunological properties of monocytes and macrophage cells. However, in the literature, studies on the effect of different stimulants on common co-stimulatory molecules of THP-1 cells are limited.

Method: In this study, we investigated the stimulation of THP-1 cells with PMA and LPS on the change in expression levels of CD70, CD80, CD86, CD252 (OX40L), CD274 (PD-L1) and CD275 (ICOSL) of co-stimulatory molecules by flow cytometry. According to our findings, CD80 and CD86 expression of classical co-stimulatory molecules increased 24 hours after PMA stimulation, but the increase after PMA-LPS stimulation was more prominent. CD70 and CD252 (OX40L) were the highest in THP-1 cells in monocyte form, whereas there was a significant decrease in LPS expression after PMA stimulation. There was no significant difference between CD275 (ICOSL) expressions but CD274 (PD-L1) expressions were significantly increased from LPS group. CD70 is a molecule that activates their activation state by interacting with the CD27 receptor in activated T and B lymphocytes. CD252 (OX40L) is an important molecule that inhibits the apoptosis of activated T and B lymphocytes such as CD70 by interacting with the OX40 molecule in T lymphocytes.

Findings and Conclusion: According to the results, we observed that both molecules decreased at 48 hours following PMA stimulation with LPS stimulation. In addition, we observed that expression of PD-L1, a critical molecule for Treg and exhausted T cell formation, increased with LPS stimulation. These data suggest that macrophages, which differ from THP-1 cells may behave differently from healthy macrophages in terms of antigen presentation. However, our data should be supported by a study with macrophages from healthy individuals.
EFFECT OF CANCER CELL LYSATE WITH STIMULATED THP-1 DERIVED MACROPHAGES ON MDA-MB 231 CANCER CELLS APOPTOSIS

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Introduction and Aim: Macrophage cells present in the cancer microenvironment both induce a natural immune response and trigger an adaptive immune response. The interaction between various cells in the tumor microenvironment effects tumor growth and progression. Macrophages derived into two main phenotypes which are M1 polarized and M2 polarized. M1 polarized macrophages exhibit antitumor, proinflammatory and cytotoxic effects, whereas M2 polarized macrophages are immunosuppressive, they promote angiogenesis, tissue repair and metastasis. In this study, the effects of macrophages on cancer cells were investigated by mimicking cancer microenvironment via preparing cancer cell lysate from apoptotic and healthy MDA-MB 231 Cancer Cells.

Method: Two types of cell lysates were prepared from doxorubicin induced apoptotic and healthy MDA-MB 231 cells. The amount of protein in these cell lysates was determined by protein analysis. Co-culture model was used to mimicking cancer microenvironment for this purpose, THP-1 human monocyte cells were cultivated in the upper part of the transwell system and differentiated with PMA. Then, MDA-MB 231 breast cancer cells were cultivated on the underside of the transwell system. LPS induced macrophage cells and non-induced were treated with cell lysates for 24-hour. After that, MDA-MB 231 cells analyzed for apoptosis and macrophages cells were stained with PE conjugated CD80, FITC conjugated CD80 and CD86 antibodies for determining macrophage polarizations by flow cytometry.

Results And Discussion: Apoptotic analysis for MDA-MB-231 by flow cytometry demonstrated that pre-apoptosis was initiated in all groups. However, higher initiation of apoptosis in MDA-MB-231 cells were determined for cell healthy cancer cells lysate. CD40, CD80 and CD86 antibody staining performed in macrophages cells results showed that the CD40 expression level were exhibited significantly higher MFI values in LPS induced macrophages cells. CD80 expression level was decreased in LPS-induced groups treated with healthy cancer cells lysate. The MFI values of CD86 expression was the increased in only non-induced macrophages cells with LPS. As a result, Macrophages cells showed increased pre-apoptotic effect on MDA-MB-231 cells. In addition, CD40, CD80 and CD86 expressions of macrophage cells treated with cancer cell lysate were increased.
A MODEL TO STUDY THE DYSFUNCTION OF THE EPITHELIAL BARRIER IN ALLERGIC RESPONSES BY TYPE I INNATE LYMPHOID CELLS

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Epithelial cells of the airway form a barrier as a first line of defense for mucosal immunity. Studies suggest disruption of barrier integrity is required to initiate mucosal immune responses (Strengert et al. 2011). Experimental evidence also point at the defective epithelial barrier function as a prerequisite to the airway inflammation in asthmatic patients (Wang et al. 2012). To overcome the challenge of studying the barrier functions in asthmatic airway inflammation, transepithelial electrical resistance models are needed to quantify the integrity of barrier dynamics in cell culture models of endothelial monolayers (Srinivasan et al 2015). Here we demonstrate a pulmonary epithelial model to study the effects of innate immune cells on the airway barrier function. We hypothesized that in a model epithelial barrier formed by human lung epithelial carcinoma cells or primary epithelial cells, soluble effector molecules from Type I innate lymphoid cells would affect the barrier function. To test this hypothesis we first cultured two human lung epithelial cell lines NCI-1299 and A549 (ATCC: CRL-5803 and CCL 185) were seeded unto xCelligence E-plates in duplicate to determine the optimal lung epithelial cell concentrations. xCelligence RTCA DP system is used for real-time electrical impedance analysis represented as cell Index. Cell Index data were also correlated with qualitative light microscopy analysis for >99% confluency. Optimal lung epithelial cell concentration were identified as 5000 cells/50 µl for a minimum of 24 hr. assay. Next, we seeded NCI-1299 and A549 at pre-determined cell concentrations that reached >99% confluency in 24 hrs. and co-cultured them with ILC1 cells seeded into the E-plate inserts with 0.4µm pore sizes. We started our co-culture experiments from 20:1 ILC1: lung epithelial cell ratios with logarithmic dilutions. Cells were monitored up to 120 hrs. and results were represented as cell Index vs. time graphs. Our preliminary results show that ILC1 cell lines would affect the model barrier formed by human lung epithelial carcinoma cells. We are further testing the barrier function of the primary human naso-bronchial epithelial cells from allergic individuals against the ILC1 cells in the barrier model we are presenting.
INTERFERON INDUCED HELICASE (IFIH1) RS1990760 POLYMORPHISM IS ASSOCIATED WITH CLINICAL SUBPHENOTYPE BUT NOT WITH LUPUS SUSCEPTIBILITY IN TUNISIA

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Background: The interferon induced helicase (IFIH1) gene, also called MDA5 or Helicard codes for the MDA5 protein (Melanoma Differentiation-Associated Protein 5) which detects double-stranded RNAs. By stimulating the production of interferons, MDA5 plays an important role in innate immunity. Some studies have reported an association between the IFIH1 rs1990760 polymorphism of IFIH1 (IFIH1 rs1990760) and systemic lupus erythematosus (SLE) as well as other autoimmune diseases such as type 1 diabetes and rheumatoid arthritis.

Herein, we aimed to assess the association of rs1990760 polymorphism of the IFIH1 and clinical manifestations in a cohort of Tunisian patients with SLE. Material and methods: The study population consisted of 141 SLE patients (13 men, 128 women, median age, 36.12 years) and 156 healthy controls. DNA was extracted from whole blood of patients and controls by salt-out method. IFIH1 SNP rs1990760 genotypes were determined by the mutagenically separated polymerase chain reaction (Ms PCR). The statistical analysis was realized by the software SPSS (18.0).

Results: Our study did not show statistically significant association between IFIH1 rs1990760 and SLE susceptibility (p = 0.33). However, this polymorphism was associated with Anti-Sjögren’s-syndrome-related antigen antibodies (anti-SSA) (p = 0.019). The dominant model analysis with the subphenotype generated similar results (p=0.02; OR=2.58, 95% CI 1.13-5.86). Furthermore, in women we found that rs1990760 in the recessive model was associated with arthritis (p=0.04; OR=0.33, 95% CI 0.33-0.95). Among 128 SLE women, 39 had arthritis. Discussion: This was the first ever study assessing the association of IFIH1 rs1990760 and SLE susceptibility, unlike other studies in Europe and Japanese population. This discrepancy between the results could be due to genetic heterogeneity between populations. Nonetheless, we demonstrate that IFIH1 rs1990760 was associated with anti-SSa antibodies and in women with arthritis. Otherwise, it would be interesting to study other SNP in the IFIH1 gene.
Introduction: Rheumatoid arthritis (RA) is a chronic inflammatory disease targeting the synovial membranes afflicting severe structural damage to cartilages and bones with both local and systemic bone loss. Osteoporosis is a potential complication of RA and affects approximately 15–36% of RA patients. Bone destruction in RA is associated with aberrant activation of osteoclasts without equivalent activation of osteoblasts. TRAF6 is a unique molecule representing a focal point for different pathways involved in bone remodeling and inflammation.

Objective: This study was aimed to investigate the association of the single nucleotide polymorphism of tumor necrosis factor receptor associated factor 6 (TRAF6), rs540386, with low bone mineral density (BMD) among patients with RA. Material and methods: TRAF6 rs540386 genotyping was performed by mutagenically separated PCR in a cohort of 188 (23 men, 165 women, median age, 56.2 years) adult RA patients and 224 age and gender-matched controls. BMD was measured using dual-energy Xray absorptiometry (DXA) (Lunar Prodigy advance scans, GE Healthcare, USA).

Results: Among the RA patients, 64 (55 women, 9 men) had low BMD comprising of 57 patients with osteoporosis and 7 with osteopenia. Whereas TRAF6 rs540386 was not associated with RA susceptibility, it was however found to be a risk factor for reduced lumbar spine Z-score in the recessive model (OR = 3.34, 95% CI = (1.01–11.00), p = 0.038). This association was confirmed further in the multivariate logistic regression analysis taking into account several potential confounding factors (OR = 3.34 (1.01–11.00), p = 0.048). In addition, mean total femur Z-score was found to be reduced in TT patients when compared to CC + CT patients (−1.30 ± 1.32 versus − 0.60 ± 1.05, p = 0.034). No association between TRAF6 rs540386 and local bone damage was observed. Conclusion. This study for the first time ever demonstrated an association between a genetic variant of TRAF6 and low BMD among patients with RA. Further investigations are needed to elucidate the exact role of this variant.
OPTIMIZATION OF EXOSOMAL TRANSCRIPTOME SEQUENCING FROM GliOBlastoma PATIENTS TO CHARACTERIZE THEIR BIO-INDICATOR CONTENT

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Introduction: Glioblastoma (GBM) is the most common primary malignant brain tumor. Despite improved treatment approaches in recent years, the patients survival time does not exceed an average of 14 months. To determine the risk of GBM, to diagnose early, to evaluate the response to treatment and to detect recurrence earlier; diagnostic, prognostic and predictive tumor markers are still needed. Tumor indicators that can be obtained non-invasively from bodily fluids such as serum and cerebrospinal fluid (CSF) would enhance the prognosis of the patients. Extracellular vesicles (exosomes-EV) are nano-sized membrane vesicles of endocytic origin by carrying lipids, DNA, mRNA, and microRNA. They are one of the key regulators between cell to cell communications and thus EV based biomarkers have a great potential in identifying disease specific indicators in vivo.

Aim: In this study via optimization of transcriptome sequencing of EVs that released into the circulation by the GBM cells we tried to determine key indicators these vesicles are transferring.

Method: Serum samples were collected from 4 GBM patients (before and after operation) and 2 healthy control. EV and RNA isolation were done with Qiagen® exoRNeasy Kit according to the protocol. EV concentrations were measured with NanoSight NS300, RNA quality were checked with Agilent Bioanalyzer Pico Kit. cDNA synthesis was done by Takara® SMART-Seq v4 Ultra Low Input RNA Kit and cDNA concentration was measured flurometrically. The sequencing libraries were prepared Nextera® XT library preparation Kit, sequenced on Illumina HiSeq 2500 system and bioinformatic analysis were done.

Findings: EV concentrations obtained from the serum ranged from 1.7 to 7.4 x 10^14 particles / ml. Isolated EVs were confirmed by Western Blot using CD81 and TSG101 antibodies. The mean amount of RNA obtained from the preoperative samples was 22.6 ± 15.5 ng (1.1-50.3 ng) and 20.9 ± 16.8 ng (1.0-52.6 ng) in the postoperative samples. The mean RNA in the control group was 21.9 ± 14.7 ng (range: 3.4-45.4 ng). RNA samples obtained from EVs were converted into cDNA and validated with two housekeeping gene (Actin, GAPDH). EV RNAs are highly fragmented compared to control total RNA, further optimization steps were necessary for library preparation. 10 ng EV-RNA is used for cDNA conversion and checked for size distribution in Bioanalyzer before library preparation. The pilot study for NGS experiments were completed for 4 patients (preop/postop) and 2 normal serum as 30 M read depth for these 10 samples.

Conclusion: During this pilot study, an efficient, robust protocol for transcriptome sequencing of scarce and degraded EV-RNA has been optimized. In the first part of the analysis differentially expressed genes between preop and normal serum has been evaluated. Heatmap and pathway analysis were done.
Several immune-cytokine related pathways were prominent in these EVs according to pathway analysis result and they should be further studied whether they can be important immunoregulators as well as biomarkers of the disease. This study will be repeated with more samples, the candidate genes will be validated with RT-PCR in the cohort in ongoing experiments.
ASSOCIATION STUDY BETWEEN TLR9 rs352140, TLR7 RS3853839 POLYMORPHISMS AND SYSTEMIC LUPUS ERYTHEMATOSUS

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Introduction/purpose: Systemic lupus erythematosus (SLE) is a complex systemic autoimmune disease. Several genes are involved in genetic susceptibility to SLE. The most recently studied are those of innate immunity including the TLR9 gene and the TLR7 gene, located on the chromosome 3 and X respectively, which encode intracellular receptors. In this study, we investigated the association of the rs3853839 polymorphisms of the TLR7 gene and rs352140 of the TLR9 gene with the LES in the Tunisian population.

Material and methods: This is a case-control study of 141 lupus and 187 healthy controls. Genotyping of this polymorphism was determined by the MS-PCR technique (mutagenically separated PCR). The frequency comparison between the two groups was established by the χ2 test.

Results and discussion: Our results indicate that the SNP rs3853839 of the TLR7 gene is not associated with lupus. In women, the G allele is present in 28% of patients and 29.4% of controls (p = 0.79). By analyzing the genotypes, we note a similar distribution in both populations. The CC genotype is found in 53.5% of the controls and in 55.1% of the patients; the GG genotype is present in 12.4% of the controls and 11% of the patients (p = 0.93). In men, the G allele is present in 23.1% of patients and 31.5% of controls. On the other hand, we found that the SNP rs352140 of the TLR9 gene was associated with SLE susceptibility. The C allele was the most frequently observed, and it was significantly more frequent among patients than controls (51.8% versus 40.17%, p = 0.003). Among all genotypes, CC was present among 34.0% of patients with SLE and 23.6% of controls (p = 0.039). In the present study, we didn't find an association between TLR7 rs3853839 and SLE susceptibility, whereas C allele of the TLR9 rs352140 polymorphism was significantly more frequent in SLE patients. Further studies on larger samples are required to confirm the association between the TLR9 polymorphisms and the lupus susceptibility.
IMMUNOGENICITY OF LEISHMANIA EXTRACELLULAR VESICLES IN COMBINATION WITH CPG ODN AS A VACCINE AGAINST CUTANEOUS LEISHMANIASIS

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Introduction: Cutaneous Leishmaniasis (CL), also known as Aleppo sore is a neglected parasitic disease that presents as self healing skin lesions or cause debilitating large chronic or recurring lesions. During 1990-2010, >46,000 new CL cases caused by 3 different Leishmania species have been identified in Turkey. Considering the vast number of refugees immigrating to Turkey from Syria where CL is highly endemic, a substantial increase in CL cases is anticipated in the near future. The absence of an available licensed vaccine coupled with the cost, toxicity and drug resistance associated with the pentavalent antimonials used for treatment, necessitates the development of an effective preventive vaccine. Herein, we explored the immune protective vaccine potential of Leishmania antigen-rich small vesicles (exosomes) secreted from parasites in combination with CpG ODN based vaccine adjuvants.

Method: Soluble leishmania antigen (SLA) or parasite exosomes were isolated from L. major. For vaccination, balb/c mice were immunized twice with SLA or exosomes in combination with CpG ODN based vaccine adjuvants and challenged with EGFP/Luciferase transgenic live parasites. In some experiments, gp63 activity of the intended vaccine was inactivated using a chemical treatment strategy. Footpad and lymph node parasite loads were evaluated using in vivo imaging. Leishmania antigen specific IgG levels were quantified from sera of immunized mice by ELISA. Th1/Th2/Th17 responses elicited by vaccine formulations were measured from antigen stimulated immunized mice splenocytes using cytometric bead array (CBA). Similar vaccine formulations were also tested as an immunotherapy approach in Leishmania-lesion established animals following their administration intraperitoneally.

Findings: Comparison of the immunization groups revealed that exosomes combined with D35 CpG ODN was the most promising vaccine formulation, inducing 63 and 77% reduction in footpad and lymph node parasite loads, respectively when compared to the unvaccinated group. Chemically inactivated exosome/D35 combination was also found to regress the parasite load when used as a systemic immunotherapeutic agent.

Conclusion: Preliminary data suggests that gp63 inactivated exosomes proved to be effective as a vaccine or as an immunotherapeutic agent against leishmaniasis when used in combination with D35 CpG ODN. This project was supported by TUBITAK grant -115S073.
ROLE OF CYTOSOLIC INNATE IMMUNE SENSING PATHWAYS IN LEISHMANIA MAJOR INFECTION

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Role of Cytosolic Innate Immune Sensing Pathways in Leishmania Major Infection

Introduction: Leishmaniasis is a parasitic disease caused by several different species of Leishmania protozoa. Depending on the infecting Leishmania species, the disease manifests as recurrent cutaneous, mucocutaneous or visceral leishmaniasis. The parasite evades and manipulates the host immune response through multiple mechanisms in a species-dependent manner. Recent evidence also suggests that Leishmania parasites infected with a dsRNA virus (Leishmania RNA virus; LRV), cause more severe disease. The lack of a potent licensed vaccine, the toxicity of the drugs used in treatment of Leishmaniasis and emergence of drug resistant/LRV positive parasites, necessitates a thorough understanding of parasite/virus detection/immune evasion mechanisms to develop better anti-Leismanial therapeutics. In this work, we focused on the role of cGAS/STING signaling pathway, NLRP3 inflammasome, and RIG-I/MAVS pathway on infection of monocyte/macrophage cell lines by Leishmania Major parasites that are LRV negative or positive.

Materials & Methods: RAW 264.7 wild-type, cGAS KO and MAVS KO cells were pre-treated or not with peptidoglycan to stimulate an M2 macrophage phenotype, and infected with LRV negative or positive, EGFP (+) Leishmania Major. Similarly, PMA differentiated THP1-Dual wild-type, cGAS KO, STING KO, STING GOF, NLRP3-deficient, ASC-deficient and TREX1-KO cells were infected with the abovementioned Leishmania strains. 24 h following infection, parasite load was determined using flow cytometry.

Results: Infection of RAW 264.7 cGAS KO cells were reduced by 50% compared to their wild-type counterparts. Infection of THP1-Dual STING-KO cells were reduced by 65% compared to their wild-type counterparts. Furthermore, the infectivity of LRV-positive Leishmania Major was around 2-fold higher than the virus negative parasites.

Conclusions: Data presented herein suggests that Leishmania Major parasites utilize the cGAS-STING-IRF3 pathway to their advantage. Furthermore, the presence of RNA virus may synergize with the cGAS/STING pathway in IRF3-activation, that somehow contributes to parasite survival/proliferation. Inhibitory drugs that target cGAS/STING and or IRF3 activation may be pivotal for the success of anti-Leishmanial immunotherapy.
ROLE OF BUTYRATE IN POST-TRANSCRIPTIONAL REGULATION OF INFLAMMATORY GENES BY REGULATING RNA BINDING PROTEINS

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Introduction: Butyrate is a short chain fatty acid that is produced by the gut microflora through fermentation of undigested starches and dietary fibers. Its important epigenetic mechanism as a histone deacetylase inhibitor (HDACi) leads to silencing of genes that are involved in cell cycle progression, apoptosis and differentiation. Additionally, butyrate is known to inhibit nuclear factor kappa B (NF-κB), a transcription factor which regulates the expression of inflammatory genes including cyclooxygenase-2 (COX-2), TNF-alpha and many others. Inflammatory response is also regulated by RNA binding proteins (RBP) that bind to the Adenylate and Uridylate rich elements (ARE) in the 3' untranslated region (3'UTR) of inflammatory genes. RBPs differ in terms of their stabilizing or destabilizing ability of target mRNAs and their localization in the nucleus or in the cytoplasm show their functionality. Butyrate has been shown to regulate many genes post-transcriptionally; however, very few studies have addressed whether butyrate can affect the localization or activity of RBPs.

Aim: We aim to show whether treatment of colon cancer cell lines with butyrate affected the expression of RBPs in order to regulate the expression of inflammatory genes post-transcriptionally.

Method: Proteins from cytoplasmic and nuclear fractions of Na-butyrate treated colorectal cancer lines (Caco2 and HT29) were analyzed by Western Blotting. The AREs from the 3'UTR of COX-2, TNF-alpha and a canonical HuR binding sequence were cloned into pGWIZ SuperNanoLuc vector and these vectors were transfected to CaCo2 cells. A luciferase assay was conducted to determine the 3'UTR activity of these sequences in the presence of butyric acid.

Findings: We observed that butyrate treatment for 48h reduced the cytoplasmic translocation of certain RBPs, especially the mRNA stabilizing protein HuR. The signaling mechanism involved decreases in the phosphorylation of proteins such as Chk2, p38 and MAPKAPK2. Moreover, butyrate suppressed the 3'UTR activity of RBP binding genes to the ARE's of COX-2, TNF and an artificially generated HuR binding sequence. This suppression was retained even when HuR was overexpressed, suggesting that in addition to its well defined role in transcriptional regulation of genes, butyrate also plays a role in the post-transcriptional regulation of inflammatory gene expression.

Conclusion: In addition to butyrate's HDACi activity, it may also reduce expression of RBPs and regulate gene expression post- transcriptionally.
INTACT MAB AND GLYCAN ANALYSIS OF BIOSIMILAR PRODUCTS BY LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY

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Introduction Recombinant monoclonal antibodies (mAbs) are safe and effective treatment options for various diseases. While original drug patents are being expired, a new door opens for producing biosimilar molecules. However, mAbs are highly complex molecules in terms of production and analysis. Evaluation of the molecular similarity of a candidate product to the originator drug is the most critical step in the development of biosimilar drugs.

Purpose The first and most important step in this context is comprehensive analytical characterization. Biosimilar candidate and reference product comparison is necessary to determine possible structural differences between the two. In this study, the molecular similarity between the reference product and the biosimilar candidate is evaluated in detail in terms of intact mAb and glycan analysis.

Method Biosimilar molecules were subjected to liquid chromatography (LC) and mass spectrometry (MS) without any sample preparation step for intact mAb analysis. Mass spectrums were obtained by UPLC-Xevo G2-XS QTOF system. Direct spectral comparison of samples for biosimilarity assessment was carried out on the UNIFI software. For analysis of post-translational modifications, glycans were enzymatically removed from the protein, labelled for fluorescence and mass detection then applied to the LC-MS system. MS data allows us to compare the relative percentage of the different glycans on both biosimilar candidate and the original molecule.

Results and Discussion Intact mAb analysis has revealed that the biosimilar product conforms to the molecular weight of the reference product. The deconvolution spectrum represented different glycoforms of the mAb that showed variation between the reference product and the biosimilar candidate. Quantitation of the main glycoforms of the molecule enables the optimization of upstream and downstream parameters in production for acquiring the desired glycan profile.

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DESIGN OF SHORT-CHAIN FATTY ACIDS PRODUCING BACTERIA AS A NOVEL THERAPEUTIC APPROACH

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Introduction: The use of short-chain fatty acids (SCFA) as inhibitors of colorectal cancer cells growth and epigenetic gene expression regulators is widely reported in the literature. SCFAs are produced by the gut microbiome and are extensively used by the colonic epithelial cells as a source of energy. Although the metabolic pathways leading to the production of SCFAs are well known, very few studies have focused on manipulating biochemical pathways in commensal bacteria to enhance SCFA production for therapeutic purposes in colorectal cancer.

Purpose: In this study, we aimed to engineer a commensal bacterium E.coli K12 to generate SCFAs in an inducible manner. The secretome of these bacteria are known to produce acetate (2-C) and propionate (3-C); however, no trace of butyrate (4-C) could be observed. Hence, we hypothesized that manipulation of biochemical pathways such as elimination of fermentation and enhanced β-Oxidation may lead to an increase in the amount of butyrate. Method: For production of butyrate in an inducible manner, we have constructed a plasmid that includes thiolase(AtoB), dehydrogenase and hydratase(FadB), reductase(FabI) and transferase(ButCoAT) in that order. Of all four enzymes, ButCoAT is the key enzyme in butyrate production pathway that was designed to participate in a reaction that generates the products for the next round of reactions in a self-feeding loop. The expression of the four His-tagged enzymes in the bacterial lysate was confirmed with Western blots. The pure His-tagged proteins were purified using a Ni-NTA column while biochemical kinetic experiments were used to determine the enzymatic activity of ButCoAT. Gas Chromatography-Mass Spectrometry (GC-MS) and HPCL were used to detect and quantify the amounts of SCFA in the secretome.

Results and Discussion: In this study, we have shown that four enzymes were overexpressed in detectable amounts. Kinetic measurements indicated the expression of a functional ButCoAT enzyme both from both crude bacterial lysate and His-tag purification of the expressed protein. Conditioned medium obtained from engineered bacteria indicated the presence of detectable amounts of SCFAs. Our results indicate that commensal bacteria can indeed be engineered to generate high amounts of SCFAs. Future studies will indicate whether the secretome rich in SCFA can change signaling pathways in colon cancer cell lines.
A NOVEL THREE-DIMENSIONAL HETERTYPIC SPHEROID MODEL FOR THERAPEUTIC TARGETING OF TUMOR-INFILTRATING MACROPHAGES

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Introduction: The complex structure of the tumor microenvironment is hard to recapitulate in vitro, particularly regarding tumor-immune cell interactions. Accordingly, it is very inefficient to monitor the usefulness of cancer immunotherapy, in vitro. This study aims to achieve better assessment of cancer immunotherapy agents with a three-dimensional (3D) heterotypic spheroid model composed of cancer cells, fibroblasts, and monocytic cells.

Methods: A 3D model in sponge-like alginate matrix (AlgiMatrixTM), as carrier material which is devoid of surface adherence, was selected to construct spheroids. THP-1 monocytic cells, CFPAC-1 pancreatic cancer cells or NCI-441 lung cancer cell, and SW982 fibroblasts were combined at co-culture ratios 2:1:1 or 1:1:1. THP-1 cells were also induced into macrophage-like cells with transient PMA stimulation. After 21 days, the matrix was cryo-sectioned, formation of the spheroids was evaluated with hematoxylin/eosin or phalloidin/DAPI staining. Size and number of spheroids were measured. The percentage and viability of myeloid cells examined with CD11b, HLA-DR markers and DRAQ7 staining.

Results: Number and size of the spheroids were increased when SW982 cells were included in the 3D cultures. These fibroblasts also supported the viability of the CD11b and HLA-DR-gated macrophage-like cells. Because of the chemical properties of the alginate, the cells more tend to generate heterotypic spheroids than the other biomaterials like collagen, matrigel.

Conclusion: Inclusion of fibroblasts in the 3D cultures may be a good strategy to provide a better environment for macrophages to communicate with cancer cells and sustain their viability. Following the optimization of this heterotypic spheroid model, novel cancer immunotherapeutics will be tested in this model.

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LAG3+HLA-DR+ HELPER T CELLS DISPLAY REGULATORY FUNCTIONS AT THE EARLY STAGES OF ACTIVATION

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Introduction: Co-inhibitor molecules such as PD-1, CTLA-4, TIM-3, and LAG3, are frequently observed on T cells as regulatory receptors in chronic inflammatory diseases and cancer. LAG3 is structurally similar to CD4 and binds MHC-II with high affinity. At certain stages of activation HLA-DR can be expressed by certain sub-populations of helper T (Th) cells. This study aims to investigate the distribution of LAG3 and HLA-DR expression through Th cells’ activation stages and to determine the functional capacities of Th sub-populations with differential LAG3 and HLA-DR expression.

Methods: Co-cultures were established with purified peripheral blood CD4+ T cells and the monocytic acute myeloid leukemia (AML) cell line THP-1 to deliver continuous costimulation whereas TCR signal was provided with anti-CD3 mAb. LAG3 and HLA-DR immunophenotyping was performed at 0, 12, 24, 48, 72, 96, 120, and 144 hours. Th cells were purified out of the co-cultures (back-sorted) according to the expression of LAG3 and HLA-DR by FACS. The influence of these Th sub-populations on cytotoxic T lymphocyte (CTL) proliferation and cytokine (IL-10, IFN-γ, IL-2) secretion was examined by eFluor670-based proliferation assay and ELISA. These cells were also visualized by HLA-DR, CD3, LAG3, IL-10 and IFN-γ immunofluorescence. Moreover, the distribution of Th cells according to HLA-DR and LAG3 expression in AML and MDS patients’ bone marrow aspirates was also determined by flow cytometry.

Results: The percentage HLA-DR and LAG3 on Th cells reached to maximal levels at 48 hours of co-culturing. The HLA-DR+LAG3+ Th cells displayed highest suppressive activity on CTL responses. These cells were positive for IL-10 and IFN-γ even though they were found to be at the early stages of T cell activation where IL-2 secretion was high. They were also detected in the bone marrow samples of AML or MDS patients.

Conclusion: Our data notes the presence of suppressive Th subpopulations which are generated at the early stages of activation, in the presence of AML cells, in vitro.
DEFECTIVE L-SELECTIN SHEDDING FROM ACTIVATED NEUTROPHILS OF ADAM17 DEFICIENT PATIENT

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ADAM17 is a membrane bound metalloprotease with wide variety of substrates including EGFR, TNFα, chemokine receptors and multiple adhesion molecules. ADAM17 deficiency is an auto-inflammatory disorder characterized with pustular dermatitis, paronychia, frequent cutaneous bacterial infections, and early onset diarrhea. In addition to clinical manifestations, loss of ADAM17 function has been linked to impaired TNFα release which may partially explain susceptibility to bacterial infections. However, pustular dermatitis and auto-inflammatory conditions observed in these patients remind the possibility of excess neutrophil infiltration to the inflamed tissue. Neutrophil transepithelial migration depends on various chemokine receptors and adhesion molecules. Cell surface expressions of some of these molecules have been known to be tightly regulated by ADAM17. For this, we aimed to investigate neutrophil function in ADAM17 deficiency. First of all, patient neutrophils were examined in terms of ROS production (DHR123) upon stimulation and found to be similar with healthy neutrophils. Additionally, activation of neutrophils with various stimuli was investigated by means of upregulation of cell surface activation marker, CD11b. Both patient and control neutrophils showed significant cell surface upregulation of CD11b following stimulation with various reagents. L-selectin (CD62L) is one of the most important adhesion molecules for neutrophil migration through vascular wall and it is considered as a substrate of ADAM17. Activation of neutrophils with various stimuli was induced rapid-shedding of L-selectin on healthy neutrophils. However, when cell surface expression of L-selectin on ADAM17 deficiency neutrophils were examined upon activation, shedding of L-selectin was significantly impaired. In other words, L-selectin down-regulation was not observed upon activation with any of the tested stimulus. ADAM17 has been also shown to regulate expression and function of CXCR2, one of the IL-8 receptor, through its sheddase activity. Since IL-8 receptors; CXCR1 and CXCR2 are known to be important for the neutrophil migration, we investigated shedding of these receptors on activated neutrophils. Down-regulation of CXCR1 on activated neutrophils was similar for patient and healthy donors which indicate ADAM17 independent regulation of CXCR1 expression. Time course monitoring of CXCR2 shedding revealed a delayed down-regulation of CXCR2 upon activation with mild stimulus. However, after prolonged activation period, cell surface expression of CXCR2 on ADAM17 deficient and healthy neutrophils was equalized. This suggested the involvement of compensatory mechanism for the regulation of CXCR2. In conclusion, ADAM17 deficiency results in the loss of regulation on L-selectin expression on neutrophils. Consequently, elevated expression of L-selectin may increase neutrophil infiltration to the site of sterile inflammation which can help to explain pustular dermatitis and other auto-inflammatory conditions observed in ADAM17 deficiency. This work has been supported by TÜBİTAK project # 315S125/315S131.
FUNCTIONAL ANALYSIS OF THE IMPAIRED IMMUNE RESPONSES IN CARD9 DEFICIENCY

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Caspase recruitment domain family member 9 (CARD9) is an adaptor protein downstream of C-type lectin receptors, including Dectin-1. Loss of function mutations in CARD9 gene impairs dectin-mediated NF-κB activation and production of Th17-inducing cytokines such as IL-6, IL-1β, and IL-23. The dysregulation in TH17-mediated immunity leads to increased susceptibility to persistent fungal infections. Furthermore, a selective neutrophil killing defect was also reported in CARD9 deficient individuals. Herein, we present data on adaptive and innate immune responses of a patient bearing a compound heterozygous mutation in CARD9 gene, prior to and following granulocyte colony stimulating factor (G-CSF) therapy.

Intracellular IL-17A and IFN-γ cytokine production from effector T-cell populations were assessed from peripheral blood mononuclear cells (PBMCs) stimulated with PMA/ionomycin using flow cytometry. T-cell specific cytokine levels were also quantitated by ELISA following PMA/ionomycin, anti-CD3/anti-CD28 or zymosan stimulation. Results revealed that PMA/ionomycin and anti-CD3/anti-CD28-stimulated CARD9-deficient patient cells produced similar levels of TH1, TH17 and Treg-associated cytokines (IFN-γ, IL-17A, IL-22 and IL-10) when compared to healthy controls. In contrast, zymosan-mediated cytokine production was severely impaired only in the patient. Similarly, pro-inflammatory cytokine production (IL-6, TNF-α and IL-1β) in PBMCs stimulated with zymosan, curdlan or heat-killed Candida albicans (HKCA) was severely impaired in the patient when compared to healthy individuals. CARD9 deficient PBMCs’ response to R848 (a TLR7/8 agonist) was comparable to healthy controls. Of note, G-CSF therapy did not improve the defective cytokine response to fungal ligands.

Neutrophil-mediated reactive oxygen species (ROS) production and neutrophil extracellular trap (NET) formation was also analysed in patient versus healthy controls. Unstimulated CARD9 deficient neutrophils had higher levels of intracellular ROS and spontaneously released NETs. However, ROS production in response to zymosan and HKCA was lower in patient’s cells than healthy controls prior to G-CSF therapy. Treatment of patient with G-CSF improved neutrophil functions (increased ROS production following zymosan stimulation) and prevented spontaneous NETosis.

Overall, these findings indicate that G-CSF therapy may improve/regulate certain neutrophil functions in CARD9 deficiency but cannot alter the observed cytokine production defect in response to fungal ligands.
TRANSCRIPTOMIC PROFILING OF HELPER T CELLS UNDER CONTINUOUS STIMULI

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Introduction: Following activation, helper T (Th) cells robustly proliferate and acquire effector functions regulated by transcriptional, epigenetic and metabolic reprogramming. Following successful clearance of foreign antigens that provoke immune responses, Th responses regress; however, in chronic inflammatory diseases, including cancer, leads to functional impairment in Th cells with a gradual loss of effector functions. This study aims to correlate functional and molecular dynamics of Th cell activation in a co-culture model wherein to isolate Th cells at distinct phases of activation and effector responses.

Methods: Naïve CD4+ Th cells were co-cultured with human monocytic cell line THP-1 in the presence of anti-CD3 mAb which was refreshed daily. Th cells’ morphology, viability (Annexin V, PI), proliferation (eFluor670 dilution assay), immunophenotype (CD3, CD4, CD45RA, CD45RO, CD62L, CCR7, CD69, CD25, CD154, TIM-3, LAG3, PD-1, and CTLA-4) and IFN-γ, IL-10, IL-2 secretion (ELISA) were analyzed at the time points 0h, 12h, 24h, 48h, 72h, 96h, 120h, and 144h. Then, these Th cells were purified out of the co-cultures by FACS and their polarization were determined actin polymerization on uniformly coated surfaces. Adhesion (xCelligence) and migration (trans-well assays) capabilities were examined. Furthermore, RNA was isolated from purified Th cell populations and RNA-seq library preparation and next-generation sequencing (NGS) was performed to reveal Th-cells’ transcriptomic profile.

Results: We were able to correlate certain features of Th cells with activation status, morphological features, surface protein expression, cell polarization, migration and adhesion, and hyporesponsiveness with a distinct transcriptomic trajectory. Especially the strong resemblance between naïve and hyporesponsive cells generated in vitro was remarkable.

Conclusion: The bio-information obtained from transcriptional signatures of Th cells at distinct activation phase kinetics can serve as a standard on biological and functional capacities of Th cells.
CIRCULATING EXOSOMES OF IDIOPATHIC NEPHROTIC SYNDROME PATIENTS HAVE IMMUNOMODULATORY EFFECTS ON HEALTHY PBMCs

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Introduction: Exosomes are nanovesicles released by virtually all known cell types under healthy and pathological conditions. They play critical roles in intercellular communication and facilitate immune modulation. Pathogenesis of idiopathic nephrotic syndrome (INS) is still unclear; one or more circulating factors or immune mediators affecting glomerular filtration barrier permeability were suspected. In this study, we examined immunological properties of plasma-derived exosomes and aimed to characterize their effects on peripheral mononuclear cells (PBMC) in childhood INS.

Material And Methods: Exosomes were isolated from platelet depleted plasma using Exosome Isolation Kit. PBMC of 16 patients (11 MCD and 5 FSGS) on relapse (taking no or low dose steroids) and 8 healthy controls (HC) were isolated by density gradient separation and characterized by flow cytometry. Healthy PBMCs were stimulated with 50 ug/ml healthies’ own (isogenic) and patients’ exosomes. IL10, IL17, IFNγ, IL4 levels were measured from supernatant by ELISA. Also intracellular staining with IL13, IL4 and IFNγ was performed to characterize B- and T-cells after exosome stimulation.

Results: Exosomes from INS patients induced significantly higher IL4, IFNγ and IL10 secretion from healthy PBMCs compared to exosomes from healthy controls. There was a significant increase in IL13 secreting CD4+ T-cells after INS exosome stimulation however there was no significant change in IFNγ secreting CD4+ and CD8+ cells or IL17 secreting CD4+ cells. CD19+ B-cell numbers were not different during relapse or remission of INS patients. PDL1+ CD19+ B-cell numbers were significantly higher in INS patients (both during relapse and remission) compared to healthy controls. But we found no increase in CD19+PDL1+ cell numbers in healthy PBMCs after stimulation with exosomes from INS patients.

Conclusions: Our data implicated that immune dysregulation in relapse INS involved both B- and T-cells. Circulating exosomes of relapse INS patients act immune modulatory on healthy and isogenic PBMCs, which suggested that they may affecting podocyte biology due to immune modulatory nature. Our current extensive studies tackle on these issues and we are attempting to unravel the immunoregulatory roles of exosomes on podocyte functions.
STIM-1 GENE MUTATIONS WITH DIFFERENT PHENOTYPES

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STIM1 is a single-pass transmembrane Ca2+ binding protein residing in the membrane of the ER. It has an ER luminal N-terminus and a cytoplasmic C-terminus, with which it responds to depletion of ER Ca2+ stores and binds to ORAI Ca2+ channels, respectively. Loss of function or null mutations of Stromal interaction molecule 1 (STIM1) are known to cause early-onset combined immunodeficiency (CID) disease with recurrent and chronic infections, autoimmunity, haemolytic anaemia, ectodermal dysplasia, muscular weakness and myalgia. STIM1 and ORAI1 form the calcium release-activated calcium (CRAC) channels and are involved in calcium signalling, which is especially important in T cells for activation, proliferation and cytokine production. In the absence of these molecules, lymphocyte stimulation was impaired although the lymphocyte count was normal. STIM-1 is also present in the sarcoplasmic reticulum, a structure similar to ER in muscle cells. This structure plays an important role in muscle contraction and relaxation by storing and releasing calcium ions. Several patients with heterozygous mutations in STIM1 and ORAI1 were recently described providing intriguing information about the pathophysiological consequences of increased SOCE. These mutations result in constitutive or increased activation of CRAC channels and are associated with a spectrum of symptoms affecting different organs and cell types. Although there is some overlap with the features of CRAC channelopathy, patients with heterozygous mutations have unique disease phenotypes. Their clinical disease spectrum ranges from non-syndromic tubular aggregate myopathy (TAM) to the more complex York platelet and Stormorken syndromes. The clinical phenotypes of the latter syndromes are very similar and dominated by platelet defects in combination with skeletal myopathy. Both syndromes can be caused by the same GoF mutation in STIM1; in addition, another GoF mutation in STIM1 causes either York platelet syndrome or TAM. All three diseases therefore appear to be part of the same clinical spectrum, with the phenotype and expressivity resulting from the mutation being determined by additional factors such as allelic variation or modifier genes. As a result of targeted next generation sequencing we found the STIM-1 mutations in 7 patients. Than we confirmed these mutations with Sanger sequencing. The clinical features and STIM-1 mutations of 7 patients will be presented. One patient has homozygous mutation in the STIM-1 gene and six of other have got the heterozygous mutation. We still investigate functional tests of the all the patients variants.
DECIPHERING IKBKE INVOLVEMENT IN HEPATOCELLULAR TUMORIGENESIS

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Introduction and Aim: Chronic inflammation, which accounts for approximately 25% of cancer-causing factors, constitutes one of the hallmarks of cancer. Throughout the course of development of this progressive disease, although the detailed mechanisms still remain elusive, inflammation is well known to have role in all phases, namely initiation, promotion, and progression. Inflammatory responses orchestrated by cancer cells and their interactions with immune cells shape a tumor microenvironment which creates a vicious circle towards poor prognosis. A better understanding of the underlying molecular mechanisms would enable discoveries of new and superior anti-inflammatory therapeutic targets. NF-κb is one of the master regulators of inflammatory pathways. It can be activated by the canonical kinase complex comprising IKKα, IKKβ and IKKγ, and/or by the non-canonical kinases namely IKKε and TBK1. The latter have been shown to be involved in many cancers. Especially IKKε seems to be an oncogene in breast and ovarian cancers. Liver cancers are well associated with inflammatory causes yet there are little known about the involvement of IKK-related kinases in liver tumorigenesis. Therefore, in this study, we aimed to decipher IKKε specific roles in liver tumorigenesis.

Methods: Bioinformatic analysis of data from patient samples in different Hepatocellular carcinoma (HCC) developmental stages reveals a correlation between IKKε expression levels and patient overall survival. To further study IKKε’s role in HCC, loss of function models were generated using gene silencing techniques and IKKε’s implication in tumor development was assessed through measuring proliferation, invasiveness and metastatic potential of IKKε depleted cells both in vitro and in vivo.

Findings: IKKε expression affects the proliferative ability of HCC cell lines both in vitro and in vivo. Additionally, expression patterns of EMT markers were altered in the absence of IKKε, suggesting its involvement in invasion and metastasis of tumor cells.

Conclusion: IKKε has plausible involvement in HCC tumorigenesis but we should better understand its role to suggest proper strategies in tackling with HCC.
ASSOCIATIONS OF INDOLEAMINE 2,3-DIOXYGENASE ENZYME ACTIVITY AND MRNA EXPRESSION WITH HUMAN PAPILLOMAVIRUS-RELATED CERVICAL PRECANCEROUS LESION

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Introduction: Genital Human Papillomavirus (HPV) infection is a common sexually transmitted infection that is usually asymptomatic but responsible for almost all cases of genital warts and cervical cancer. Most HPV infections regress spontaneously, and the virus clearance is usually attributed to an effective immune response. However, in almost 10% of cases, the virus persists and progresses to precancerous and cancerous lesions. Indeed, HPV allows cancer cells to escape immune surveillance. Therefore, understanding mechanisms that promote this immune escape could contribute to the development and of effective therapies against these infections. One of the immune evasion mechanisms is the expression of indoleamine 2,3-dioxygenase (IDO), an immunosuppressive enzyme that plays a key role in infection diseases and cancer progression. IDO catalyzes the first and rate-limiting step in the conversion of the tryptophan (Trp) into kynurenine (Kyn) along the Kyn pathway.

Aim: We first conducted a molecular epidemiological study to investigate prevalence of HPV among cervical-screened unvaccinated Tunisian women. We then aimed to assess, for the first time, the activity and expression of IDO in HPV-infected women.

Method: A total of 300 women were included in this study and are divided into: women with squamous intraepithelial lesion (SIL, n=67) and no intraepithelial lesion (NIL, n=233). HPV-DNA detection was carried out on cervical samples by real-time polymerase chain reaction. Plasma Trp and Kyn concentrations were measured by High-performance Liquid Chromatography (HPLC) method with ultraviolet (UV) detection and the IDO enzymatic activity was determined by the kyn/trp ratio. IDO mRNA expression levels in epithelial cervical cells was evaluated using quantitative real time PCR (RT-qPCR) method.

Findings And Conclusion: The overall HPV prevalence was 35.7% with significantly higher prevalence among women with SIL than those with NIL; 71.6 % and 25.3% respectively. To understand the immunosuppressive mechanisms associated with HPV infection and SIL occurrence, the activity and expression of IDO was investigated. Our results show that analysis of the correlation between the plasma IDO enzymatic activity and its cervical expression shows a significant positive correlation (r = 0.218; p = 0.038). In addition, the activity and expression of IDO were significantly higher among HPV-positive women and SIL women compared to HPV-negative women (p = 0.005 and p = 0.023; respectively). These results suggest that IDO is upregulated in HPV-positive women and is associated with the cervical precancerous lesions occurrence.
COMPONENT RESOLVED DIAGNOSIS IN HYMENOPTERA VENOM ALLERGY

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Background Hymenoptera venom allergy is an IgE-mediated hypersensitivity reaction to Hymenoptera venoms. Occasionally, patients are not aware of the insect type that they have been exposed to and such uncertainties in history cause many problems related to diagnosis. In this study, the contribution of component resolved diagnostics (CRD) were evaluated in patients who had a systemic reaction due to a Hymenoptera. Method Eighty-one patients from 5 different centers were included in the study. Prick, intradermal skin with venom extracts were performed and serum specific IgE levels for whole venoms were measured. sIgEs for Api m1, Api m2, Api m10, Ves v1, Ves v5 were evaluated by venom allergen components by immunoblot method (Dpd-Dx Venom kit 2).

Results Seventeen out of 33 patients with bee venom allergy revealed a positive skin test result and/or a high sIgE level to honeybee venom whereas 16 patients had positivity with both venoms. In 11 out of 17 patients with bee venom allergy, the diagnosis was confirmed with CRD whereas CRD was negative in the remaining 6 patients. In 13 of the bee allergic patients with double positivity to both venoms (13/16), double sensitivity was confirmed with CRD. CRD revealed a sensitivity of 73% in bee venom allergic patients. Seven of 18 patients with wasp venom allergy demonstrated sensitivity only to Vespula spp according to skin tests and/or sIgE levels whereas 11 patients revealed double positivity. Total sensitivity of Ves v1 and Ves v5 was calculated as 88%. Eight of 20 patients with a history of hypersensitivity to both venoms showed double sensitivity with CRD, one patient revealed cross-reactivity, seven patients was found sensitive only to bee venom, and finally one patient was sensitive only to Vespula spp. 10 patients were uncertain for the culprit insect type and half of them had double sensitivity and one had cross-reactivity according to CRD.

Conclusion CRD seems to be more helpful in diagnosing wasp venom allergy than bee venom allergy. It also gives information to differentiate double sensitivity from cross-reactivity and in cases where the culprit insect is unknown.
THE GENOTOXIC EFFECTS of STI571 AND GENISTEIN ON CHRONIC MYELOBLASTIC CELLS

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The aim of this study is to assess genotoxic effects of STI571 and genistein when used alone and combined on K562 cells. Chronic Myeloid Leukemia (CML) is a clonal myeloproliferative disease resulting from neoplastic transformation of pluripotent hematopoietic stem cells. More than 90% of CML patients have Philadelphia chromosome (Ph) is formed by t(9;22)(q34;q11), the reciprocal translocation between chromosome 9 and 22. Ph chromosome is specific cytogenetic finding in almost all CML patients. This resulting translocation adds the 3'abl gene found on chromosome 9q34 to the 5'bcr gene segment on chromosome 22q34 and the bcr-abl fusion gene is generated. The fusion gene encodes a tyrosine kinase which activates intracellular signaling pathways such as AKT, ERK and STAT. STI571 (imatinib), a type II protein kinase inhibitor, is used in the targeted therapy of CML. It binds competitively to the ATP-binding domain of the BCR-ABL protein. STI571 causes apoptosis in hematopoietic cells expressing bcr-abl. Studies have shown that STI571 can cause genotoxic damage at high doses when used alone or combined with other chemotherapeutic agents. Genistein is an isoflavone found in soybean and is a tyrosine-dependent protein kinase inhibitor. It blocks the growth of cancer cells by inhibiting protein kinase-mediated signaling mechanisms and induces cell differentiation. Genistein also inhibits cell proliferation and angiogenesis. Genistein is a natural tyrosine kinase inhibitor that exerts anti-cancer effect by inducing G2/M arrest and apoptosis. K562, human CML cells were grown in RPMI 1640 medium containing 10% FBS at 37 °C and in a humidified incubator. All experiments were repeated at least three times. Cell viabilities were determined by trypan blue exclusion test. The quantitative colorimetric method MTT cell proliferation assay was used. After determination of IC50 values, STI571 and genistein were administered to the cells alone and combined for 24 hours. Untreated cells were used as control group. Checkerboard microplate synergy test was applied to cells for determination of STI571 and genistein interactions. The dilution of STI571 (A) were made horizontally and the dilution of genistein (B) vertically in a microtiter plate in 100 µL. The cells were distributed to each well in 50 µL (1X104 cells). The cells were incubated at 37°C in a CO2 incubator. Interaction was evaluated according to the following equations: FICA=IC50A in combination / IC50A alone FICB=IC50B in combination / IC50B alone where FIC is the fractional inhibitory concentration. For the determination of genotoxic damage, alkaline SCGE was performed. Electrophoresis was performed at high pH on slides. After electrophoresis, slides were stained with ethidium bromide and scored under a fluorescence microscope. A minimum of three SCGE slides were prepared for each treatment and 100 nuclei were blindly scored per slide. Nuclei were scored as 0, +1, +2, +3 and +4 by a blinded observer according to apparent relative proportion of DNA in the tail and head. p<0.05 was considered statistically significant. STI571 induced genotoxic damage and when combined genistein its effectiveness was increase. It was concluded that genistein may be a suitable candidate for combination therapies.
IgE PRODUCTION IN CHRONIC GRANULOMATOUS DISEASE RELATED TO FUNGAL ANTIGENIC STIMULATION

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Introduction: Chronic granulomatous disease (CGD) is an inherited disorder of phagocytes in which affected patients suffer from recurrent bacterial and fungal infections. It is defined as the inability to create a normal respiratory burst in response to microbial stimulus. We observed that high serum IgE formation can be detected in some of the CGD patients during diagnosis or follow up. However, the cause of higher IgE production is not well known.

Aim: In this study, we aimed to investigate the effect of in vitro antigenic stimulation of Aspergillus and Candida on IgE class-switch recombination from naive B cells in CGD patients.

Method: CGD patients (n=10) and healthy controls (n=7) were included to the study. Serum total IgE, specific IgE and skin prick test were conducted. Naive B cells were isolated and incubated for 7 days with the following conditions: (i) Unstimulated media (cRPMI+ naive B cells), (ii) Naive B cells+CD40L+HA-peptid+IL-4+IL-21 (IgE media), (iii) Naive B cells+CD40L+HA-peptid+IL-4+IL-21+Heat-Aspergillus, (iv) Naive B cells+CD40L+HA-peptid+IL-4+IL-21+Heat-Candida. After 7 days, culture supernatants were collected and IgE production was measured by ELISA.

Findings: When compared to healthy controls, in vitro IgE production was statistically higher both in conditions (ii) and (iii) (p=0.035 and p=0.032, respectively) in CGD patients. When CGD patients were evaluated separately, both Aspergillus (condition iii) and Candida (condition iv) stimulations exhibited increased IgE production compared to IgE media (p=0.0002 and p=0.038, respectively). In addition, in CGD patients, IgE production with Aspergillus stimulation was significantly higher than Candida stimulation (p=0.0003). In healthy controls, in vitro IgE production in condition (iii) was also higher compared to condition (ii) (p=0.0006).

Conclusion: In CGD patients, chronic exposure to Aspergillus could be associated with high IgE production. This work was supported by Marmara University research project BAPKO No: SAG-A-110915-0410.
NATURAL KILLER CELL ACTIVITY IN WHOLE BLOOD AND PURIFIED NK CELLS

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Natural Killer Cell Activity in Whole blood and purified NK cells Nilgün Akdeniz, Esin Aktas Cetin, Gunnur Deniz

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Background: Natural killer (NK) cells serve a major role in the innate and adaptive immune responses against tumor transformation or pathogen-infected cells. NK cells exert natural cytotoxicity to eliminate malignant cells without prior sensitization or class I MHC restriction. Furthermore, NK cells stimulate the adaptive immune response by secreting proinflammatory cytokines to counteract the escape mechanisms promoted by tumor cells. On the basis of the relative densities of CD56 surface expression, NK cells can be split into 2 major subsets as CD56dim and CD56bright NK cells. CD56dimCD16bright NK cells comprise 90% of peripheral blood NK cells, have a high cytolytic capacity, and secrete low levels of cytokines, whereas CD56brightCD16- NK cells secrete a great number of cytokines but acquire cytotoxicity only after prolonged activation.

Purpose: To investigate the cytotoxicity capacity of NK and also cytotoxic T cells (CD3+CD8+), purified NK and whole blood cells were stimulated with promoca (a proprietary formulation engineered recombinant cytokine that specifically activates NK cells) and their functions were analyzed by flow cytometry.

Method: NK cell functions were performed in 5 healthy adult volunteers. Peripheral blood mononuclear cells (PBMC) were isolated and NK cells purified by FACSAnia Il. Purified NK and whole blood cells were stimulated with promoca at 37°C in 5% CO2 incubator for 24 hours. The content of intracytoplasmic interferon-g (IFN-gama), interleukin (IL-10) and lysosomal-associated membrane protein-1 (LAMP-1 or CD107a) expression of NK and CD8+ T cells were analyzed by flow cytometry. IFN-g levels were also evaluated by sandwich enzyme-linked immunosorbent assay (ELISA) in stimulated and unstimulated culture supernatants. Statistical analyses were performed by student t test.

Results: Stimulation of purified NK and whole blood cells with promoca significantly increased the intracytoplasmic IFN-g levels (p<0.05) in CD3- CD56dimCD16bright NK cell population, however there was no stimulation in CD8+ T cell subset. Degranulation capacity of CD3- CD56dimCD16bright NK and CD8+ T cells were analyzed by CD107a expression by flow cytometry, and only NK cells showed significant increased by promoca stimulation (p<0.05). In contrast to IFN-g, intracytoplasmic IL-10 levels did not show any significant difference when the purified NK and whole blood cells were stimulated with the same stimuli.

Conclusion: NK cells are activated by the binding of NK cell activating receptors with activating ligands on target cells. Proinflammatory cytokines such as IL-2, IL-12, IL-15 and IL-18 are also able to activate NK cells. Activated NK cells induce cell death of target cells through secreting perforins and granzymes, engaging death receptors on target cells, and secreting IFN-g. The cytokines induce proliferation and maturation of NK cells, promote cytokine production, and enhance NK cell cytotoxicity. Whole blood and purified NK cells stimulated with promoca showed increased IFN-g release. Similar to IFN-g secretion, degranulation capacity of NK cells was also elevated. In contrast to NK cells, promoca stimulation did not affect CD8+ T cell cytotoxicity.
IMMUNOSCORING OF EARLY STAGE NON-SMALL CELL LUNG CANCER; DETERMINATION OF SUPPRESSOR AND CYTOTOXIC LYMPHOCYTE POPULATIONS AND THEIR CYTOTOXIC MECHANISMS

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Introduction. Lung cancer is the most common cause of cancer-related death; approximately 85% are non-small cell lung carcinoma (NSCLC). Deficiencies in anti-tumor immunity play a major role in carcinogenesis of NSCLC. Natural killer (NK) cells are cytokine producing innate lymphoid cells exhibiting anti-tumoral functions. Predominance of CD4+CD25+FoxP3+ regulatory T (Treg) and CD8+CD28− cytotoxic T (Tc) cells exerts regulatory population capable of inhibiting anti-cancer immune response.

Purpose. In order to determine immune scoring, the ratio of circulating lymphocytes, suppressor and cytotoxic T cell subsets and cytotoxic mechanisms of NK and CD8+ T cells were investigated.

Method: Forty-six newly diagnosed NSCLC patients and 46 healthy subjects were enrolled to this study. The stage of NSCLS was T1-3N0M0 and none of the patients had preoperative chemotherapy or radiotherapy. CD3−CD16+CD56+ NK cells, CD4+CD25+Foxp3+ regulatory (Treg) and CD8+CD28− T cells were analyzed in peripheral blood by flow cytometry. Cytotoxic capacity of NK and CD8+ T cells were determined by CD107a degranulation assay.

Results. The ratio of total CD3+ T and CD4+ T helper cells were lower in NSCLC group (p=0.020, p=0.021, respectively), but HLA-DR expression in CD3+ T cells was also higher in NSCLC group (p<0.001). There was no significant difference for Treg cells between the groups (p=0.067). There was also no significant difference for CD8+ T cells (p=0.33), but among them CD8+CD28- suppressor cytotoxic T cells were increased (p=0.001) while effector CD8+CD28+ cytotoxic T lymphocytes were decreased in NSCLC (p=0.004). Both CD3−CD16+CD56+ NK and CD3+CD16+CD56+ NKT cells were increased in NSCLC (p=0.002 and p=0.005, respectively). Cytotoxic activity of NK cells remained unchanged between the groups (p=0.299), but the cytotoxic activity of CD8+ T cells was increased in the NSCLC group (p=0.005). When we compared the patients who had lymph node metastasis and who did not, only the number of CD3+HLA-DR+ activated T cells was higher in patients with Lymph node involvement (p=0.005). We further compared early stage (T1) and late stage patients (T2-3), conversely the number of CD3+HLA-DR+ activated T cells was lower in late stage patients (p=0.035).

Discussion. Our results demonstrated that the balance of cytotoxic T cells was skewed towards a regulatory CD8+CD28- T cells, favoring immunological escape of tumor tissue. T cells had a less activated phenotype in late stage patients compared to early stage patients, which might be responsible for a higher tumor growth in these cases. Lymph node involvement might be inducing T cell activation, as activated T cell numbers were higher in the group with lymph node involvement. There was no functional impairment that predisposes tumor growth, in NK or NKT subsets. The role of immune cells in different stages of cancer needs to be further studied.
DECREASED LEVELS OF Akt AND p-Akt1 IN PERIPHERAL CD4+CD25+CD127-/LOW REGULATOR T CELLS OF MULTIPLE SCLEROSIS PATIENTS

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Introduction: Multiple sclerosis (MS) is an autoimmune disease characterized by demyelination, axonal loss and irreversible neurodegeneration induced by infiltration of auto-reactive immune cells into the central nervous system (CNS). Auto-reactive T cells and antibodies are believed to have a pathogenic role in inflammation and tissue damage in the CNS. The Akt/PKB molecule is thought to contribute many cellular functions such as transcriptional regulation, nutrient metabolism, cell growth and survival. Akt/PKB signaling pathway also has an important role in T cell development and survival. Increased or decreased activation of Akt is the basis of the pathophysiology of various complex diseases including Type-2 Diabetes mellitus and cancer. There are studies showing the loss of CD4+CD25+CD127-/low Regulator T (Treg) cell functional suppression in patients with MS.

Purpose: The relationship between the levels of the Akt molecule, especially of phosphorylated-Akt (pAkt) molecule in Treg cells, and the severity of the disease was investigated.

Method: Two different patient groups with clinically isolated syndrome (CIS, n=8) and relapsing remitting multiple sclerosis (RR-MS, n=10) were selected. Age and sex matched healthy individuals (n=20) were also included. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood samples of subjects by ficoll concentration gradient centrifugation method. Anti-CD4 PerCP, -CD25 APC-Cy7, -CD127 PE-Cy7 monoclonal antibodies were used to identify the Treg cells. After stimulation for 15 min with PMA/Ionomycin intracellular staining was performed to evaluate the Akt1 (Ser473) and p-Akt levels in Treg cells and other T cell subgroups. Data were collected and analyzed with FACS DiVa software and FACSAnia II flow cytometer.

Results: Akt1 and p-Akt ratios were measured in CD3+ (T cells), CD8+ (T cytotoxic), CD4+ (T helper) and CD4+CD25+CD127-/low Treg cells. We report a significant decrease in the p-Akt levels of peripheral blood Treg cells in patients with CIS (p= 0.0017) and RR-MS (p= 0.0332) in comparison with healthy donors. The total number of attacks (p= 0.063), EDSS (p= 0.047) and progression index (p= 0.025) of the patients with the Akt1 ratio of Treg cells were found to be correlated.

Discussion: Akt activity determines the effector T cell response development against Tregs, suppressive function of Tregs, and also inducible Treg (iTreg) development. It has been shown that Akt inhibits Foxp3 expression by phosphorylating Foxo-1/Foxo-3a. However, the exact role of Akt isoforms in T cell responses has not been determined yet. The molecular mechanism by which Akt-1 and Akt-2 differentially controls the proliferation of Tregs is currently under investigation. Our findings indicate that pAkt levels are decreased in Treg cells of MS patients as early as the CIS stage of the disease, thus it can be used to predict the severity and prognosis of the disease. However, in order to correlate the results with the severity of the disease, other Akt isoform levels in MS patients should also be considered in further studies.

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Determination of Effects of BTB-ZF Transcription Factors in Cancer

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Introduction BTB-ZF proteins are evolutionarily conserved transcription factors that repress target gene expression. In the human genome, 49 genes are responsible for encoding these proteins, which are involved in a range of physiological functions such as immune system development, fertility, skeletal and neurological development. Mutations in the members of this protein family are associated with different types of cancers, especially lymphomas.

Aims and Methods: In this study, we aimed to clone the BTB domains of select proteins of the family. We have started to express these proteins in E.coli and started to purify the proteins by affinity and size exclusion chromatography. The purified proteins have been used to determine the rules of homo and heterodimerization as well as interactions with co-repressors such as Ncor/Bcor/SMRT by using Biacore surface plasmon resonance (SPR). Moreover, we aimed to generate GFP and RFP fusion BTB proteins to identify interacting BTB domains in BHK cells by fluorescence imaging which is known as F2H (Fluorescent Two-Hybrid) assay in short. Finally, we aimed to generate mutations in the genes encoding these proteins by CRISPR/Cas9 genome editing to identify their significance in cancer development.

Keywords: Cancer, Transcription Factor, CRISPR/Cas9 genome editing, BTB-ZF proteins
DIMINISHED CELL NUMBER AND FUNCTION OF NATURAL KILLER CELLS IN LRBA DEFICIENT PATIENTS

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Lipopolysaccharide-responsive beige-like anchor (LRBA) deficiency is a rare genetic disorder of the immune system. The most common clinical features are immune deficiency, autoimmunity and lymphoproliferation. Natural killer (NK) cells play a major role in the rejection of both tumors and virally infected cells by cytotoxic function and producing cytokines. There are two subgroups of NK cells, including cytokines-producing CD56bright and CD56dim with cytotoxic capacity. Recent reports suggested that LRBA might have a role in receptor signaling by immune effector cells, but the studies are limited NK cell number and its receptors in these diseases. In this study, peripheral blood NK cell number and functions of LRBA deficient patients were investigated by flow cytometry. Samples obtained from two LRBA mutant siblings (male; 5 and 11 years) and three healthy children (male; 6, 8, 16 years). NK cytotoxicity receptors (NKp30, NKp44, NKp46, and NKG2D) and intracellular (perforin and granzyme) molecule expressions were analyzed in lymphocyte, CD3-CD16+CD56- and CD3-CD16-CD56+ NK cell subsets by flow cytometry. CD3-CD16+CD56- and CD3-CD16-CD56+ NK cells percentage were decreased in LRBA deficient patients compared to healthy children. In contrast to elevated NKG2D, HLA-DR, perforin and granzyme expressions in lymphocytes, CD3-CD16+CD56- and CD3-CD16-CD56+ NK cell subsets, NK cytotoxicity receptor expression in lymphocytes of LRBA deficient patients were decreased. Diminished NK cell number in LRBA deficient patients, but increased HLA-DR and NKG2D expression indicate that NK cells are more active. Increased perforin and granzyme expression might be a sign of high cytotoxic capacity of LRBA deficient patients but might not secrete these enzymes. In conclusion NK cells might have a role in PID clinic.

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Key words: NK cells, LRBA Deficiency, Flow cytometry
IN BEHÇET’S DISEASE INFLAMMATORY STATUS MIGHT DIRECT ILC CELLS TO ILC3+ SUBSET

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Innate lymphoid cells (ILCs) are lymphoid cells that do not express rearranged receptors and have important effectors and regulatory functions in innate immunity and tissue remodeling. Uncontrolled activation and proliferation of ILCs can contribute to inflammatory autoimmune diseases. Behcet’s disease (BD) is a complex systemic inflammatory disorder, in which the role of ILCs is still unknown. In this study, the quantity and effect of ILCs in the pathogenesis of BD were investigated. Peripheral blood mononuclear cells were isolated from whole blood from forty three BD patients (32 active and 11 inactive stage) and thirteen healthy subjects. ILCs were stained by using anti-Lineage, -CD45, -CD161, -CRTH2, -NKp44, -c-kit and -CD127 monoclonal antibodies and analyzed by flow cytometry. ILC3 and NKp44- ILC3 cells were increased in all BD patients compared to healthy subjects (p= 0.05 and p= 0.04, respectively), whereas NKp44+ ILC3 cells were decreased (p= 0.03). Similarly, ILC3 and NKp44- ILC3 cells were increased (p= 0.01 and p= 0.05, respectively). NKp44+ ILC3 cells were decreased (p= 0.04) in active patients compared to healthy subjects but not in inactive patients. There was no significant difference between patients and healthy subjects regarding ILC1 and ILC2 subgroups. Recent studies showed that increased neutrophilic infiltration and IL-17 secreting Th17 cells in BD. It is known that ILC3+ cells are similar to Th17 subset as cytokine variety and transcription factors, regarding our data showed that inflammatory status in BD might direct ILC cells to ILC3+ subset, especially NKp44+ ILC3 cells.

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THE EFFECTS OF PERIPHERAL MACROPHAGES ON MICROGLIA INFLAMMASOMES PATHWAY AFTER LPS STIMULATION IN CELL CULTURE

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Introduction and Aim: There is a bidirectional crosstalk between resident microglia and bone marrow derived macrophages (BMDM) in central nervous system inflammation. Studies have shown that BMDM infiltrating the brain after an injury suppress the proinflammatory cytokines produced by resident microglia. BMDM regulate microglia mediated inflammation and form thereby a natural control mechanism against the detrimental effects of excessive inflammation after injury. In this preliminary study, the relationship between microglial inflammasomes pathway and the antiinflammatory effects of BMDM after LPS stimulation, was evaluated.

Method: Primary microglia and BMDM have been isolated from the brains and long bones of adult C57BL/6 mice, respectively (n=3). Cells were co-cultured for 4 hours after stimulation with LPS and been washed for 20 hours in serum-free medium. Genes associated with the inflammasomes pathway were evaluated using the commercial kit for RT-qPCR. Gene expressions were calculated as “CT values” and their up- or downregulations have been evaluated as fold changes.

Findings: LPS stimulation increased the IL-1, IL-6 and TNF-α gene expression and this increase was suppressed in accordance with the literature after coculture with BMDM. LPS related increase in microglial NLRP3 expression was suppressed in coculture. When subgroup genes associated with the NFkB pathway involved in the production of all three cytokines were examined; b1 and bia were found positively and bib was found negatively correlated with increase in cytokine gene expression, but these changes were independent with the BMDM coculture. Microglia and BMDM coculture caused significant changes in expression of TGF-β activated kinase 1/MAP3K37 binding protein (Tab) 1 and 2, Fas-associated via death domain (FADD) and cathepsin B genes.

Conclusion: According to the results of this preliminary study, the suppressive effect of the BMDM on the microglial production of IL- 1 after LPS stimulation is regulated in the signal 1 process of the canonical inflammasomes pathway. This regulation takes place at the Tab and FADD steps. The expression of Tab genes, which are known to act by modifying intracellular p38MAPK localization, is negatively correlated with proinflammatory cytokines, and is associated with FADD expression. This result suggests that these genes may have a driving effect on microglia polarization rather than cell survival in inflammation.
TH17-INDUCING CONDITIONS LEAD TO IN VITRO ACTIVATION OF BOTH TH17 AND TH1 RESPONSES IN BEHCET'S DISEASE

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Introduction And Aim: Interleukin-17 (IL-17) has been associated with the pathogenesis of various autoimmune/inflammatory diseases. The aim of this study was to investigate the expression of Th17-related immunity in an innate immunity-dominated vasculitis, Behcet's disease (BD).

Method: Peripheral blood mononuclear cells from 37 patients (age: 38.5 ± 9.8 years) with BD, and 25 healthy controls (HC) (age: 39.1 ± 9.3 years), were cultured in Th17-inducing conditions (IL-6, Phytohemagglutinin (PHA), IL-1β, and IL-23) for 6 days. Cultured cells were stained with CD4, CD8, CD3, TCR gamma/delta, CD19, interferon-γ (IFN-γ), and IL-17 antibodies to determine the intracellular cytokine secretion by flow cytometry.

Findings: IL-17 expression by CD8+ and γδ+ T cells was higher in BD compared to HC (p = 0.004, p = 0.003, respectively). No differences were observed between the groups in the IL-17 production by B cells. Under Th17-inducing conditions, production of IFN-γ by CD4+, CD8+, and γδ+ T cells was also higher in BD compared to HC (p < 0.05 in all).

Conclusion: Our results suggest that under Th17-stimulating conditions, T cells express both IL-17 and IFN-γ in BD. More prominent IL-17 and IFN-γ production by all lymphocyte subsets in BD might be associated with the increased innate responses, early tissue neutrophil infiltrations and late adaptive immunity in BD.
HUMAN PAPILLOMAVIRUS (HPV) VIRAL LOAD IN THE PLASMA OF TUNISIAN WOMEN WITH NORMAL CYTOLOGY AND CERVICAL LESIONS

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Introduction: Genital HPV infection is one of the most common sexually transmitted infection in the world. Several types of HPV can infect the genital tract, especially types HPV-6, -11, -16 and -18. Low-risk non-oncogenic HPV are responsible of acuminated condylomas (HPV 6 and 11) and high-risk or oncogenic HPV are responsible of the development of precancerous and cancerous lesions (HPV 16/18).

Aim: The aim of this study was to detect and quantify HPV DNA in the plasma of Tunisian women with no intraepithelial lesion (NIL) and women with cervical lesions (CL) using real-time quantitative PCR assay. Method Human plasma and cervical samples were collected from 45 non-vaccinated women attending the National Office of Family and Population of Monastir and the Center of Maternity and Neonatology of Monastir for regular gynecologic control. PCR using MY09/MY11 degenerate primers were used for HPV-DNA detection in cervical samples. Plasma circulating viral load quantification was performed using consensus primers GP5+/6+ in a real time qPCR. Findings Overall, 51 % (23/45) of cervical samples and 93% (42/45) of plasma samples were positive for HPV-DNA. The rate of HPV detection in both cervical and plasma samples increased with cervical cytology results, ranging from 27% in NIL women to 59 % in women with CL. The plasma circulating viral load median level were higher among CL women compared to NIL women ( 6121 vs 2857 copies /ml respectively) . Moreover, among CL women, cervical cancer patients showed the highest plasma circulating viral load median level compared to precancerous lesions patients and NIL women (15255, 5676, 2857 copies /ml respectively , p= 0.038 ). Conclusion Results obtained in our study confirm that HPV DNA can be detected and quantified in plasma samples of women with asymptomatic cervical infection. Moreover, plasma HPV-DNA might be a valuable marker for monitoring the disease progression to cervical cancer.
REGULATION OF NEUTROPHIL FUNCTIONS BY EFFECTOR TH1 CELLS

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Introduction: Neutrophils critically contribute to innate immune responses, nevertheless how these cells interact with helper T (Th) cells needs to be better investigated. The aim of this study is to examine the influence of in vitro generated effector Th1 cells on neutrophils in the context of activation, viability, phagocytosis and reactive oxygen species (ROS) production capacities.

Methods: CD4+ T cells were collected from healthy volunteers' PBMC by MACS and co-cultured with monocytic cell line THP-1 under anti-CD3 stimulation for 72h. After confirmation of effector Th1 phenotype (proliferation, IL-2 and IFN-g secretion and loss of early activation markers CD69 and CD154) at the effector phase, the Th cells were collected out of the co-cultures with FACS back-sorting. Neutrophils freshly isolated from peripheral blood were brought together with these back-sorted Th cells at various ratios. At 0h, 12h, 24h, and/or 48h and 72h, phagocytosis was assayed with latex-bead capture assay; ROS production index was measured with DCFDA; CD62L and CD66b levels were determined for neutrophil activation and viability (PI staining) was measured by flow cytometry.

Results: The viability of neutrophils were supported by the presence of Th1 cells. However, ROS production capacity of neutrophils was decreased as the amount of CD4+ T cell ratio was increased in the co-cultures. These cells influenced the phagocytic activity of neutrophils. In terms of activation markers, the loss of CD66b and CD62L tend to decrease as the amount of Th cells increased in the co-cultures at 12h and 24h.

Conclusion: Our preliminary results indicate the functional regulation of neutrophils when they encounter with Th1-oriented cells at the effector phase.
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Myasthenia gravis (MG) is an autoimmune disease in which autoantibodies play an important role in the pathogenesis. In most patients, autoantibodies occur against the acetylcholine receptor (AChR). Tfh cells are helpers for B cell proliferation, isotype switching and long-term antibody response in the secondary lymphoid tissue. Cytokines secreted from CD4+ T cell may play a role in B cell antibody production in the periphery. The aim of this study was to assess differences in cytokine profile in peripheral CD4+ T cells and Tfh-like cell subgroups between MG and healthy controls (HC). Patients with clinically diagnosed MG and anti-AChR antibodies (AP-MG) were included. AP-MG patients (n=72) were grouped as immunosuppressive therapy positive (AP-MG-ISP (n=27)) and negative (AP-MG-ISN (n=45)). Patients with disease onset earlier than 50 years of age (EOMG, n=40) and later (LOMG, n=32) were also evaluated separately. Age and sex-matched 43 donors were included as HC. Mononuclear cells from blood samples were stained with CD4 (PeCy7), CXCR5 (FITC), CCR6 (PE) and CXCR3 (Efluor 660) antibodies. Tfh cells described according to the differential expression of the chemokine receptors on CD4+CXCR5+T cells: CXCR3+CCR6− (Tfh1), CXCR3−CCR6− (Tfh2), and CXCR3−CCR6+ (Tfh17). Intracellular expression of IL-21, IL-4, IL-17, IL-10, IFN-γ in CD4+ T cells were measured by flow cytometry after stimulation with PMA/ionomycin for 4 hours. Tfh cells, their subgroups and cytokine levels were compared between groups by the non-parametric tests. IL-21, IL-4 and IL-17 productions in CD4+ T cells were increased in AP-MG patients compared to HC (median values: IL-21 = 1.6 vs 0.3%, p < 0.0001, IL-4 = 1.8 vs. 1.0%, p < 0.0001 and IL-17 = 1.4 vs. 1.0%, p = 0.092). LOMG patients produced higher IL-17 than the EOMG patients (p = 0.018). IL-21 and IL-4 production were increased both in EOMG (p < 0.0001, p < 0.0001) and LOMG patients (p < 0.0001, p = 0.001). Immunosuppressive treatment only increased IL-10 production of AP-MG-ISP patients compared to AP-MG-ISN (1.6 vs. 0.9%, p = 0.005) without any other further effects. CD4+CXCXR5+T cell population was lower in AP-MG as in AP-MG-ISN and AP-MG-ISP groups than HC (median values: 5.1% vs. 12.3%, p = 0.003, AP-MG-ISN =6.6%,p = 0.022, AP-MG-ISP = 3.4%, p = 0.003). Both EOMG and LOMG patients had lower levels CD4+CXCXR5+T cells (p = 0.006, p = 0.012). Based on chemokine receptors on these cells, Tfh2 cells of AP-MG (median values: 51.3%) and of all subgroups such as EOMG, LOMG, AP-MG-ISN, AP-MG-ISP patients were higher than HC (36.8%, p = 0.011, p = 0.020, p = 0.033, p = 0.041, p = 0.021). IL-4 and IL-21 as regulating cytokines of antibody production, as well as IL-17 have been shown to be effective in the periphery in this organ-specific disease development. Increase in Tfh2 population and IL-4 in patients suggest Tfh2 cells as a relevant subgroup in pathogenesis. This study is supported by TÜBİTAK (116S317).
CD4+CD8+HLA-DR+ T CELLS IN HEALTHY HUMAN THYMUS

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Introduction: Thymus is the primary lymphoid organ responsible of T cell development and central tolerance. The presence of class II MHC-expressing cells in the thymic tissue is well established. On the other hand, in the periphery, T cells at certain phases of activation can also upregulate HLA-DR. This preliminary study aims to immunophenotypically characterize the HLA-DR positive thymocytes found in the healthy thymus.

Methods: Control thymus tissues were obtained from young individuals, homogenized and incubated with collagenase-II. Cell suspensions were filtered and separated by 1,077 g/ml Ficoll density gradient. Immunophenotyping was performed with the antibodies reactive with TCR, CD3, CD4, CD8, HLA-DR, CD28, CD62L, CD69, CD154, CCR7, CD124, CD127, CD25, IFNGRA, CD45RA, CD45RO, and CD20. T cell populations were also purified by FACS and labeled with CFSE, stimulated with PMA-ionomycin or concanavalin A, and proliferation was assayed by flow cytometry. These thymocyte subpopulations were also subjected to cytological examination by May Grünwald-Giemsa staining under light microscope.

Results: HLA-DR was expressed on a sub-population of CD4+CD8+ thymocytes which was positive for IFNGRA, CD69, CD62L, CD45RA, CD45RO, CD28, and CD3. Moreover, these markers were higher on the HLA-DR+ fraction when compared to that of HLA-DR-negative CD4+CD8+. These cells did not differ morphologically and they did not display proliferative capacities.

Conclusion: Our studies are going on with functional characterization and assessment of differentiation status of CD4+CD8+HLA-DR+ thymocytes.

This study is supported by Hacettepe University Research Fund (TSA-2017-15499).
MUTATED CCDC124 GENE; A SUGGESTED INDUCER OF HODGKIN LYMPHOMA REED-STERNBURG CELLS PHENOTYPE

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Introduction and Aim: Coiled coil domain containing protein-124 (Ccdc-124) is a centrosomal protein for which the precise function is not determined yet. It is found to be translocated to the midbody region during the cytokinesis stage of the cell cycle; where it is suggested to play a role in the physically separated of the two postmitotic sister cells by severing the intercellular bridge in a process that is known as cytokinetic abscission. Previously we showed that a CRISPR/Cas9 induced mutation of the ccdc124 gene in HEK293T cells resulted in failure of cytokinesis and formation of large multinucleated cells. Observed under live cell imaging microscopy, these mutant cells attempt to divide but rapidly re-fuse and form large cells; a phenotype characteristic of Hodgkin Lymphoma (HL) Reed–Sternberg cells. Therefore we aimed to investigate the role that this gene might play in the development of HL.

Method: two Hodgkin Lymphoma cell lines (L428 and HDLM2) were selected for DNA sequencing of the Ccdc124 gene. Additionally, mutant Ccdc124 gene was over expressed in wild type HEK293T cells and Hela FUCCI cells to identify if the mutant phenotype could be dominantly transferred. Findings: DNA sequencing of the Ccdc124 gene in HL cell lines revealed the presence of multiple SNPs and mutations which might be correlated to the development of the large, multinucleated cell phenotype. Over expression of mutant Ccdc124 in wild type HEK293T cells or Hela FUCCI cells also resulted in a multinucleated cell phenotype.

Conclusion: We determined an association between mutations in the Ccdc124 gene, cytokinesis failure and lymphoma development.
MEDIASTINAL LYMPH NODE REMOVAL IMPROVES NATURAL KILLER CELL DYSFUNCTION IN NON-SMALL CELL LUNG CANCER

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Introduction. Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related death globally. We have previously demonstrated that patients who had preoperative staging with video-assisted mediastinoscopic lymphadenectomy (VAMLA) had better clinical outcome. However, the exact mechanism of the mediastinal lymph node removal in cancer treatment outcome was unclear.

Purpose. The aim of this study was to evaluate the effect of mediastinal lymph node removal on NK function in systemic blood, and compare with preoperative blood and lymph nodes.

Methods. NSCLC lung cancer patients (n=21, mean age=64 ± 7), undergoing preoperative mediastinal staging by VAMLA were included. Peripheral blood mononuclear cells were isolated from tumor draining lymph nodes (TDLN) and peripheral blood. Activatory NKG2D, DNAM-1 and NKP46 and inhibitory NKG2A receptors, PD-1 and CTLA-4 molecule expressions, intracellular IL-10, TNF-α & IFN-gamma levels were analyzed in cytokine secreting and cytotoxic NK cell subsets. Cytotoxic capacity of NK cells was investigated by granzyme A secretion and CD107a-based degranulation assay by flow cytometry. Serum levels of sPD-L1, sPD-1 and CTLA-4 were measured by ELISA.

Results. The ratio of cytokine-secreting NK subset (CD56bright/dimCD16-) increased in blood after VAMLA (p=0.02), while other NK subsets remained relatively unchanged. Cytotoxic NK cell subsets (CD16brightCD56dim and CD16bright/CD56-) were diminished in TDLN compared to blood (p<0.001), while cytokine-secreting subset remained relatively unchanged. PD-1 and CTLA-4 expressions in blood NK cells were reduced in all NK cell subsets after VAMLA (p<0.05). In TDLN, PD-1 and CTLA-4 expressions were significantly higher in cytokine-secreting NK subset (p<0.05). Expression of NKG2A was also reduced in all NK subsets after VAMLA (p<0.01), while NKG2D and DNAM-1 expressions were increased only in cytotoxic NK subset (p<0.05). DNAM and NKG2D molecule expressions were reduced in TDLN, while NKG2A was higher in TDLN (p<0.05). After VAMLA, IL-10 secreting NK cells were significantly reduced among CD56+ NK cells (p=0.001), while TNF-α and/or IFN-gamma secreting cells remained unchanged. In TDLN, IL-10 secreting cells were significantly higher (p<0.001), and TNF-α and/or IFN-gamma secreting cells were significantly lower compared to blood (p<0.001). After VAMLA, CD107a expression increased significantly in K562 stimulated cytotoxicity assays (p=0.042), while unstimulated levels remained unchanged. In the lymph node samples, NK cells demonstrated lower CD107a expression in K562 stimulated cytotoxicity assays compared to peripheral blood (p<0.001). However, there weren’t any significant changes in plasma levels of sPD-1, sPD-1 and sCTLA after VAMLA (p>0.05) and compared to healthy controls (p>0.05), which probably fails to represent the above-mentioned functional changes in cellular level.

Conclusion. This study has shown for the first time, that mediastinal lymph node removal ameliorated both cytotoxic and cytokine secreting functions of NK cells.
We have also demonstrated that NK cells in lymph nodes had an immunomodulatory phenotype and reduced cytotoxicity in tumor draining lymph nodes, which probably contributes to NSCLC pathogenesis. Removal of mediastinal lymph nodes eliminates this functional influence and improves anti-tumor responses of NK cells.
EFFICACY OF A NOVEL LYP-1-CONTAINING SELF-MICROEMULSIFYING DRUG DELIVERY SYSTEM (SMEDDS) FOR ACTIVE TARGETING TO BREAST CANCER.

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Introduction: An ideal cancer therapy targets the tumor cells selectively without damaging healthy tissues. Even though the tumor-specific markers are limited, these molecules can be used for the delivery of anti-cancer drugs as an active targeting strategy. Since the lymphatic system plays a critical role in the dissemination of cancer cells, the drugs directed through lymphatics can feasibly reach to the sites of metastasis. Lyp-1 is a peptide that binds to the p32 receptor which is highly expressed not only on the lymphatic endothelium but also on the malignant cells; thus, making this peptide ligand a preferable candidate to mediate active targeting of lymphatics and cancer cells. In this study, different formulations of Lyp-1 containing lipid-based nanopharmaceautics so-called self-microemulsifying drug delivery systems (SMEDDS) were developed and tested for their efficacy in targeting breast cancer.

Materials And Methods: In vitro anti-cancer efficacy of Lyp-1 and/or Dox HCl either in solution or in the formulations was tested on MDA-MB-231 and 4T1 breast cancer cells. The amount of viable cells was assessed with 3-(4,5-di- methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. 4T1 cells were incubated with the Lyp-1 labelled with a fluorescent probe (Lyp-1-FAM) and the median fluorescence intensity (MFI) values were measured utilizing a flow cytometry. RT-PCR was performed to confirm the expression of p32 in 4T1 cells and in the tumor tissues established in mice. The protein levels of p32 in the MDA-MB-231 and 4T1 cell lines and in the 4T1 tumors established during in vivo studies were assessed by Western Blot. In vivo experiments performed with 4T1 Tumor-bearing mice. formulations were administered twice a week by intraperitoneal route. Geometric mean of the tumor diameters was measured for 4 weeks. Histopathological changes and metastatic foci were evaluated in the tumors, mesenteric lymph nodes, lungs, and liver samples. The 4T1 tumor-bearing or healthy (control) mice were intraperitoneally injected with Lyp-1-FAM solution in PBS or the Lyp-1-FAM in the SMEDDS formulation. Peripheral blood, peritoneal fluid, liver, mammary and mesenteric lymph nodes, and tumor tissue samples were evaluated for biodistribution of formulations.

Results: Following the selection of non-toxic formulation, doxorubicin hydrochloride and Lyp-1 were co-administered in the SMEDDS, which resulted in a significant increase in in vitro cytotoxicity in p32-expressing breast cancer cells, 4T1 and MDA-MB-231. Accordingly, the uptake of Lyp-1 in the SMEDDS by the cancer cells was demonstrated. The expression of p32 was detected in the 4T1 tumor tissues which were efficiently targeted with Lyp-1 in the SMEDDS. When doxorubicin was co-administrated with Lyp-1 in SMEDDS via intraperitoneal administration, tumor growth and metastasis were significantly reduced.

Conclusions: Stable lipid-based drug delivery systems (SMEDDS) with droplet size under 100 nm have been developed, and the potential of these novel drug delivery systems to combat tumor growth in breast cancer has been shown in tumor bearing mice. Lyp-1 and Dox HCl have been successfully co-administrated in the same delivery system without any further chemical process. This approach could be applicable for the delivery of other anticancer drugs.
IL-15 EXPOSURE REDUCES TH17-ASSOCIATED CYTOKINE PRODUCTION BY HUMAN MONOCYTE DERIVED DENDRITIC CELLS

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Aim: Dendritic cells (DCs) are critical for presentation of microbial and tumor antigens to T cells. In this study, we aimed to assess the impact of long term and short-term IL-15 cytokine exposure of human monocyte-derived dendritic cells on the generation of Th17 cell polarizing cytokines exclusively IL-23 by DCs.

Materials and Methods: PBMCs were purified via Ficoll Paque from peripheral blood of healthy donors. Monocytes were magnetically selected via CD14 Miltenyi beads and differentiated into DCs with GM-CSF and IL-4 for 5 days in the presence or absence of IL-15 (100ng/ml) for long term exposure experiments. Then DCs were matured with LPS, PGN, anti-CD40 or curdlan for 1 day. For short term exposure experiments, IL-15 was added only during maturation of DCs. DCs generated in these conditions were characterized with respect to the expression of MHC and costimulatory molecules on the cell surface, production of cytokines IL1B, IL-6, IL-23p19, IL-12, TGFB via flow cytometry or real time qPCR or ELISA. Finally, phosphorylation of signaling molecules after curdlan stimulation were assessed by phospo-flow based assays.

Results: Short- or long-term exposure of DCs to IL-15 suppressed IL-23 production. As a result, IL-15-exposed DCs suppressed IL-23 production. Importantly, we observed a reduction in the surface Dectin-1 receptor levels by IL-15-exposed DCs. In line with these observations, curdlan stimulation resulted in reduced phosphorylation of p38, ERK1/2, NF-kB p65 ve AKT by human DCs exposed to IL-15 compared with controls. These results may explain why IL-15-exposed DCs produce less IL-23 after maturation with curdlan, which is a ligand of Dectin-1. Conclusions: Exposure to IL-15 of human DCs during their differentiation or maturation programs DCs against Th17 polarization.

Keywords: IL-15, Th17, Dendritic cell
**NKT10 AND NKTFH CELLS REPRESENT TWO INDEPENDENT iNKT CELL SUBSETS THAT CAN COUNTER-REGULATE EACH OTHER**

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**Introduction:** Invariant natural killer T cells (iNKT) are memory-like T cells with an invariant T cell receptor alpha chain. iNKT cells recognize CD1d-presented glycolipids and can be activated by α-galactosylceramide (αGalCer). After activation with αGalCer, iNKT cells rapidly produce cytokines, like IFN-γ and IL-4. Similar to CD4+ T helper cells, mouse iNKT cells can be divided into several distinct subsets. Whereas NKT1, NKT2, NKT10, and NKT17 cells develop in the thymus, others, such as NKT10 and NKTFH cells expand/arise in vivo after immunization of mice with a strong, Th1-biasing antigen. These subsets are defined by the expression of particular transcription factors and cytokines.

**Method:** We stimulated C57BL/6 control mice in vivo with using of 2 ug of alpha-GalCer. Bcl6-expressing NKTFH cells are detected 3-14 days after in vivo activation. In contrast, IL-10 producing NKT10 cells are prevalent in vivo 14 days to 4 months after activation. However, when we checked Bcl6-deficient mice, we found that NKT10 cells can differentiate and even more NKT10 cells develop in these mice.

**Aim:** Due to this kinetics, NKT10 cells could derive from NKTFH cells, however, their relationship has not been investigated so far.

**Findings:** Here, we demonstrate, using Bcl6-deficient and C57BL/6 control mice, that NKT10 and NKTFH cells are functionally independent. NKT10 cells could not only differentiate in Bcl6-deficient mice, their numbers were even increased.

**Conclusion:** These data indicate (i) that NKT10 cell differentiation is independent of NKTFH cells and (ii) that NKTFH differentiation impairs NKT10 cell expansion in mice. These finding suggest that NKT10 cells could be increased in vivo for therapeutic applications by inhibiting Bcl6, for example for the treatment of autoimmune diseases.
TUMOR-MACROPHAGE CROSS-TALK IN CO-CULTURE: CHANGES OF PROLIFERATION PROFILES

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Introduction: Tumors are very diverse and contain numerous subpopulations of cells. We employed RAW 264.7 macrophages to study the interactions between laryngeal cancer cells and macrophages. RAW 264.7, a murine macrophage cell line, can be polarized into either M1 or M2 macrophages. Interactions of RAW 264.7 macrophages with human cancer cells such as lymphoma and breast cancer cells have been reported in previous studies. In the present study, to resolve better the interactions between laryngeal cancer cells (HEp-2) and macrophages (RAW 264.7), we performed an in vitro non-contact co-cultivation system to analyze the influence of microenvironment on both tumor (HEp-2) and macrophage cells (RAW 264.7). The aim of this study was to explore the impact of co-culture of laryngeal cancer cells (HEp-2) and macrophage cells (LPS-activated or none) on vitality and growth of both cells in vitro by biochemically using MTT based cell viability assay and by dynamically using real time cell analysis assay (i.e. xCELLigence RTCA DP).

Materials and Methods: To mimic the physiological environment where cancer triggers macrophage infiltration, we investigated the effect of conditioned medium (CM) from HEp-2 RAW 264.7 cell cultures on microenvironment of the tumors. For indirect co-cultivation system, we prepared conditioned medium (CM) from RAW 264.7 macrophage cell cultures (LPS-activated or none) to mimic the environment around the cancer cells. RAW 264.7 cells were plated in DMEM containing 10% FBS and allowed to attach overnight. The supernatants were collected at the specific time points, centrifuged to remove cell debris, and called as RAW264.7CM or LPS RAW264.7CM to culture HEp-2 cells. We also prepared conditioned medium (CM) from HEp-2 cell cultures to mimic the environment of the macrophage cells in tumors called as HEp2CM to culture RAW 264.7 cells. After 24, 48, 72 hours of CM treatment, cell viabilities were assessed using MTT assay. For co-culture experiments with impedance measurements, the death and detachment of cultured cells was recorded as a decrease in cell index (CI) following CM treatments by xCELLigence RTCA DP instrument (ACEA Biosciences, San Diego, CA). The comparisons between experimental groups were performed using unpaired two-tailed Student’s t-tests. The statistical analysis was performed with GraphPad Prism version 6 (GraphPad Software, San Diego, CA, USA). A P value less than 0.05 were considered statistically significant.

Results and Discussion: In this coculture model, RAW 264.7 cells were cultured in Hep2-CM medium to stimulate cancer-related changes in the macrophages. The control group was defined as 0% Hep2-CM, i.e., cell culture without conditioned medium. After being cultured in Hep2-CM in the absence of LPS stimulation for 24, 48, 72 h, the growth of RAW264.7 cells were analyzed. The cell viability was decreased significantly (p < 0.05) compared to that in the control medium. In addition to that, both RAW-CM and the LPS-RAW-CM models induced cell death of laryngeal cancer cells (HEp-2) with more noticeable effect of LPS treatment.

Conclusions: Macrophages contribute to different aspects of cancer progression such as cancer cells proliferation, survival, invasion, metastasis and angiogenesis. These results suggest that survival of laryngeal cancer cells (HEp-2) and macrophages (RAW 264.7) in
UNDERSTANDING THE ACTIVITY AND REGULATION OF IMMUNO BIOLOGY IN DLBCL CELL LINES

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Activation induced cytidine deaminase (AID) is a DNA-specific enzyme which converts cytidine to uracil. After first exposure with antigens, B lymphocytes enter somatic hypermutation and class switch recombination reactions in germinal center. Generally, AID activity is higher in B cells in germinal center whereas low in mature B cells. AID enzyme is mainly active in Ig gene regions but also may lead to DNA fractionation in non-Ig genes such as BCL6, BCL7, MYC, mir-142 and PAX5 which directly effect the B cell lymphoma genesis. In the literature it is shown that AID expression level is higher in ABC-DLBCL than GCB-DLBCL. So the somatic hypermutation activity is expected to be higher in ABC-DLBCL but it is found higher in GCB-DLBCL. It may be because of the different regulation factors in ABC-DLBCL and GCB-DLBCL.

Hypothesis AID expression level is higher in ABC-DLBCL cell lines than GCB-DLBCL in contrast to lower somatic hypermutation rate.

Aims
To confirm the higher expression level in ABC DLBCL cell lines than GCB DLBCL cell lines
To confirm the higher somatic hypermutation level in GCB DLBCL than ABC DLBCL by GFP assay
To understand targeting and off-targeting of AID
To detect the AID recruiting factors
To understand the mutation mechanisms of AID

Methods
The AID transcript levels in ABC-DLBCL (Hbl1, OciLy3, Rck8, Riva, Sudhl2 and Tmd8), GCB-DLBCL (Carnaval, Dlbcl1, Farage, Mieu, Nudhl1 and Sudhl6) and Burkitt Lymphoma cell lines (Ramos and Ramos AidKo) were detected in reference to housekeeping TBP gene by qPCR method. The AID transcript level in Ramos cell line was set to 1. In the next step, somatic hypermutation activity in those cell lines was investigated by GFP assay and flow cytometry. To detect the somatic hypermutation activity, the Super Divac vector which contains enhancer regions for AID was transferred into cells. After transfection, GFP (+) cells were sorted and incubated for 30 days. The AID enzyme was activated with the help of enhancer regions in cells which integrated Super Divac vector into their genomes. Due to somatic hypermutation activity of AID, GFP expression became negative. Those cells which were infected by the No Divac vector (lack of enhancer regions) were used as negative control.

Findings:
Transcript levels of Aid is higher in ABC-DLBCL cell lines than GCB-DLBCL cell lines. Somatic hypermutation is higher in GCB-DLBCL cell lines than in ABC-DLBCL cell lines.

Conclusions
AID expression in ABC cell lines is higher than in GCB in average. Infection and plating of most of the cell lines including Dlbcl1, Farage, Nudhl1, Sudhl8, Hbl1, OciLy3, Rck8, Sudhl2 is difficult. Carnaval and Mieu from GCB, Riva and U2932 from ABC has the most stability to further implement our experiments. Out of seven ABC cell lines, Riva and U2932 has the most stability to further implement our experiments. Out of seven ABC cell lines, Riva and U2932 is the best plating and infection efficiency. In Riva and U2932 cells, there is no hypermutation most likely due to absence of AID. We would like to test this by transferring AID into these cell lines.
STING MEDIATED INNATE IMMUNE SENSING IN IN VITRO MODELS OF STING-ASSOCIATED VASCULOPATHY WITH ONSET IN INFANCY (SAVI) AND AICARDI-GOUTIÈRES SYNDROME (AGS)

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Introduction Nucleic acid sensing mechanism operate in response to pathogen invasion and the ensuing elevated type I IFN response is terminated following clearance of the pathogen. Recently, a group of diseases, characterized by sustained type I IFN-mediated sterile inflammation have been classified as "type I interferonopathies". Here, we investigated the impact of dysregulated signaling in two interferonopathies, STING-associated vasculopathy with Onset in Infancy (SAVI) and Aicardi-Goutières syndrome (AGS) using in vitro cell line models. Specifically, type I IFN secretion from cold-stress induced versus uninduced STING M155V or N154S expressing cells were analyzed to delineate the mechanism behind the cold-induced exacerbation of inflammation observed in SAVI patients. Similarly, TREX1 KO THP cells were used as a model of Aicardi-Goutières syndrome. Type I IFN suppressing activity of various inhibitors were tested in both models. Finally, we also investigated whether or not cGAS/STING signaling is regulated by circadian rhythms.

Methods To delineate spontaneous type I IFN producing intracellular signal transduction mechanism of SAVI pathology, disease-associated human gain-of-function variant of STING M155V (STING M155V) encoding plasmid was stably transfected into STING KO B16 Blue cell line. Basal levels of type I IFN production and STING phosphorylation was assessed using wild type STING (STINGwt), STING knockout (STING-/-) and mutant STING (STING/M155V) expressing B16 Blue IFN reporter cells at different cell densities, various cold-exposure conditions and/or in the presence of inhibitors. Similarly, TREX-deficient IFN reporter THP-1 monocytes were used as an in vitro model of AGS. To assess possible circadian rhythm associated fluctuations in cytosolic DNA recognition pathways, spontaneous or ligand-stimulated type I IFN production and NF-κB activation in WT, STING-/-, cGAS-/-, TREX-/- and STING N154S Dual THP cells were analyzed subsequent to circadian rhythm synchronization with 0.1 µM dexamethasone at specified intervals for a period of 48 hours.

Results And Discussion I) Data indicate that cold-induced type I IFN exacerbation in SAVI in vitro model stems from upregulation in STING expression and consecutive STING phosphorylation.

II) Experiments with specific inhibitors suggest that chronic type I IFN production depend more on the STING/TBK1/IKK pathway rather than the autocrine/paracrine type I IFN-mediated JAK/STAT pathway in AGS model. Furthermore, Amlexanox was the most effective inhibitor capable of reducing type I IFN signature by 80% in this model.

III) Our preliminary experiments suggest that cytosolic nucleic acid stimulated-type I IFN production might be regulated by circadian clock and STING might play a role in this regulation. Our results also indicate that for AGS and SAVI, type I IFN secretion period and amplitude might be affected by light to dark and dark to light transitions. Based on the timing of oscillations, optimal inhibitor application time might impact the therapeutic utility and therefore management of disease symptoms.