MIMIC-VI

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TURKISH SOCIETY OF IMMUNOLOGY

6th INTERNATIONAL MOLECULAR IMMUNOLOGY & IMMUNOGENETICS CONGRESS (MIMIC-VI)

April 28 - 30, 2025

ACIBADEM UNIVERSITY

ISTANBUL, TÜRKİYE

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ABSTRACT BOOK

6th INTERNATIONAL MOLECULAR IMMUNOLOGY & IMMUNOGENETICS CONGRESS (MIMIC-VI)

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SCIENTIFIC PROGRAM

MONDAY, APRIL 28th, 2025	
09:00 – 10:30	**REGISTRATION**
10:30 – 11:30	**OPENING CEREMONY** Opening Speeches by the Host and Organizers Moderators: **Tolga Sütlü, İhsan Gürsel**
11:30 – 12:30	**KEYNOTE LECTURE** Moderators: **Günnur Deniz, Barbaros Oral** Human T cells: differentiation, defense, and regulation **Federica Sallusto** *ETH Zurich, CH*
12:30 – 13:00	**CONGRESS PHOTO SHOOT (MAIN HALL STAGE)**
13:00 – 14:00	**LUNCH BREAK**
14:00 – 15:30	**IMMUNE RESPONSE TO INFECTION** Moderators: **Mayda Gürsel, Akif Turna**
14:00 – 14:30	Host-Plasmodium interactions: One step closer to malaria vaccines **Cevayir Çoban** *The University of Tokyo, JP*
14:30 – 15:00	Emerging Infections and Immunology **Önder Ergönül** Koç University, TR
15:00 – 15:15	**OP-01** Investigation of SARS-CoV-2 Nucleocapsid and Spike N501Y Variant-Specific IgG Subtypes in Convalescent Pediatric Patients from COVID-19 Using Multiplex Flow Cytometric Bead-Based Assay **Mehmet Karaçay** *Istanbul Technical University, TR*
15:15 – 15:30	**OP-02** Development and Evaluation of the Efficacy of a Novel Monoclonal Antibody Targeting CX3CR1 for Pain Relief in Pancreatic Cancer, Chronic and Acute Pancreatitis **Deniz Baybağ** *Acibadem University, TR*

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MONDAY, APRIL 28th, 2025	
15:30 – 16:00	**SATELLITE SYMPOSIUM** SARTORIUS From Concept to Cure: The Role of Platform Development in Cell Therapy **Érica A. Schulze**
16:00 – 16:30	**COFFEE BREAK**
16:30 – 18:30	**CANCER IMMUNOLOGY AND IMMUNOTHERAPY** Moderators: **Güneş Esendağlı, Ceren Çıracı**
16:30 – 17:00	Insights into NK cell cytotoxicity – how to select the best (serial) killer for cancer therapy? **Carsten Watzl** *Technical University Dortmund, DE*
17:00 – 17:30	mRNA based cancer therapeutics **Mustafa Diken** *Johannes Gutenberg University, DE*
17:30 – 18:00	Biological sex at the intersection of cancer-neuro-immune crosstalk **Defne Bayık** *University of Miami, USA*
18:00 – 18:15	**OP-3** End-to-end Vector and Process Development for Clinically Applicable CD19-targeted CAR-T Cell Production in Türkiye **Betül Çakıcı** *Acibadem University, TR*
18:15 – 18:30	**OP-4** Characterization of tumor-infiltrating CD66b+ monocyte subset discloses NF-kB-mediated differentiation **Hamdullah Yanık** *Hacettepe University, TR*
18:30 – 19:00	**COFFEE BREAK**
19:00 – 21:00	**TSI GENERAL ASSEMBLY MEETING**

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TUESDAY, APRIL 29th, 2025	
09:00 – 10:30	**IMMUNE SYSTEM IN HEALTH AND DISEASE** Moderators: **Arzu Aral, Batu Erman**
09:00 – 09:30	Unveiling the molecular basis of T cell malfunctions and disorders using quantitative interactomics **Bernard Malissen** *Centre d'Immunologie INSERM-CNRS, FR*
09:30 – 10:00	The intersection of innate and adaptive immunity in the lung: implications for chronic pulmonary insufficiency **Sidonia Fagarasan** *RIKEN, JP*
10:00 – 10:15	**OP-5** Asparagine availability controls germinal center B cell homeostasis **Yavuz F. Yazıcıoğlu** *University of Oxford, UK*
10:15 – 10:30	**OP-6** Investigating the tumor immune microenvironment through innovative data analysis interfaces **Atakan Ekiz** *İzmir Institute of Technology, TR*
10:30 – 11:00	**SATELLITE SYMPOSIUM** BD FACSDiscover™ A8, The First Spectral Cell Analyzer with Real-Time Imaging **Tim Schenkel**
11:00 – 11:30	**COFFEE BREAK**
11:30 – 13:00	**MOLECULAR MECHANISMS IN AUTOIMMUNITY** Moderators: **Güher Saruhan Direskeneli, Eda Tahir Turanlı**
11:30 – 12:00	Immunological memory – a roadblock to regeneration of tolerance in autoimmunity **Andreas Radbruch** *Charité – Universitätsmedizin Berlin, DE*
12:00 – 12:15	**OP-7** Innate lymphocyte activation and interferon-driven pathways in Behçet's disease skin lesions **Atay Vural** *Koç University, TR*
12:15 – 12:30	**OP-8** Phosphorylation of signaling molecules in peripheral and thymic B cells and responses to T follicular helper cell-related cytokines in myasthenia gravis **Cemre Aktaş** *Istanbul University, TR*
12:30 – 12:45	**OP-9** CD22 Defines a Core Follicular Regulatory T Cell Subset that Controls the Antibody Response **Fatma Betül Öktelik** *Istanbul University, TR*

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TUESDAY, APRIL 29th, 2025	
12:45 – 13:30	**BALLOON RELEASE EVENT IN CELEBRATION OF "DAY OF IMMUNOLOGY" & "WORLD PRIMARY IMMUNODEFICIENCY WEEK"**
13:30 – 14:30	**LUNCH BREAK**
14:30 – 16:30	**INBORN ERRORS OF IMMUNITY** Moderators: **Yıldız Camcıoğlu, Ahmet Özen**
14:30 – 15:00	Immunoregulatory Functions of DOCK/STAT Complexes **Talal Chatila** *Boston Children's Hospital, USA*
15:00 – 15:30	Targeted Therapies in Inborn Errors of Immunity **Elif Karakoç Aydıner** *Marmara University, TR*
15:30 – 16:00	Inborn errors of immunity research in Türkiye: Results from a specialized research center "Can Sucak Lab" **Baran Erman** *Hacettepe University, TR*
16:00 – 16:15	**OP-10** Therapeutic Use of Bone Marrow-Derived Tolerogenic Dendritic Cells Modified by Lentiviral Transduction in CIA Model **Gözde Arslan** *Bursa Uludağ University, TR*
16:15 – 16:30	**OP-11** B and T Lymphocyte Subgroups in Patients with Selective IgA Deficiency **Semra Demir** *Istanbul University, TR*
16:30 – 17:00	**COFFEE BREAK**
17:00 – 18:00	**DAY OF IMMUNOLOGY LECTURE** Moderator: **Günnur Deniz (EFIS President-elect)** The immunology of sickness metabolism caused by a viral infection **Bojan Polic** *EFIS President. University of Rijeka, HR*
18:00 – 20:00	**POSTER SESSION**

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WEDNESDAY, APRIL 30th, 2025	
09:00 – 10:30	**EXOSOMES AND THE IMMUNE SYSTEM** Moderators: **İhsan Gürsel, Sinem Öktem Okullu**
09:00 – 09:30	A fresh look at classic immunology through the perspective of extracellular vesicles **Edit Buzas** *Semmelweis University, HU*
09:30 – 10:00	Dendritic cell derived extracellular vesicles for immune therapy of cancer **Susanne Gabrielsson** *Karolinska Institutet, SE*
10:00 – 10:15	**OP-12** Leishmania infantum Extracellular Vesicles Mediate Protection from Visceral Leishmaniasis and Long-Term Cross Protection from Cutaneous Leishmaniasis **İsmail Cem Yılmaz** *İzmir Biomedicine and Genome Center, TR*
10:15 – 10:30	**OP-13** Immunological Profiling Following SARS-CoV-2 Vaccination **İrem Evcili** *İzmir Biomedicine and Genome Center, TR*
10:30 – 11:00	**SATELLITE SYMPOSIUM** Advice and Advancements for the Flow Cytometric Analysis and Sorting of Extracellular Vesicles **Owen Hughes**
11:00 – 11:30	**COFFEE BREAK**
11:30 – 13:00	**EMERGING TECHNOLOGIES IN IMMUNOLOGY** Moderators: **Tolga Sütlü, Uğur Sezerman**
11:30 – 12:00	**EMBO Young Investigator Lecture** Physics of cells as a measure of immune cell functional states **Erdinç Sezgin** *Karolinska Institutet, SE*
12:00 – 12:30	Tissue-resident memory T cells identified in the muscle of patients with myositis using single-cell RNA sequencing **Begüm Horuluoğlu** *Karolinska Institutet, SE*
12:30 – 12:45	**OP-14** Discovery of Tumor-Specific T Cell Receptors Through Single-Cell Sequencing of Tumor-Infiltrating Lymphocytes in Melanoma **Elif Çelik** *Acibadem University, TR*
12:45 – 13:00	**OP-15** Investigating alternative polyadenylation sites in tumor-associated macrophage differentiation at single-cell resolution **Ebru Kocakaya** *Ege University, TR*

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WEDNESDAY, APRIL 30th, 2025	
13:00 – 13:30	**SATELLITE SYMPOSIUM** Advancing Immunology with 10X Genomics: Single-Cell & Spatial Innovations **Bio Koen De Gelas**
13:30 – 14:30	**LUNCH BREAK**
14:30 – 16:30	**FUTURE VACCINES AND IMMUNOTHERAPIES** Moderators: **Ayça Sayı Yazgan, Selim Badur**
14:30 – 15:00	Chemical switch between A-B-Z form nucleic acids for the in situ Immunotherapy **Ken J. İshii** *The University of Tokyo, JP*
15:00 – 15:30	Advancing Cancer Immunotherapy: The Emerging Role of Engineered NK Cells. **Rafet Başar** *MD Anderson Cancer Center, USA*
15:30 – 16:00	Intercellular metabolic crosstalk as an immunotherapy target in brain tumors **Dennis Watson** *University of Miami, USA*
16:00 – 16:15	**OP-16** Enhancing the Breadth of Protection from Neisseria Meningitidis Infection via Bivalent OMV Vaccine **Zeynep Arzum** *İzmir Biomedicine and Genome Center, TR*
16:15 – 16:30	**OP-17** Production of nanobodies targeting IL-1R1 (interleukin-1 receptor type 1) **Gizem Çile** *Acibadem University, TR*
16:30 – 17:00	**COFFEE BREAK**
17:00 – 17:45	**IŞIL BERAT BARLAN AWARD CEREMONY** Moderators: **Günnur Deniz, Güher Saruhan Direskeneli**
17:45 – 18:30	**CLOSING CEREMONY**

Oral Presentation

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[OP-01]

Innate lymphocyte activation and interferon-driven pathways in Behçet's disease skin lesions

Saba Khoshbakht[1], Özgür Albayrak[2], Ergün Tiryaki[1], Orhan Ağcaoğlu[3], Ayşe Öktem[4], Gizem Pınar Sun[5], Elif Er Gülbezer[6], Sümeyre Seda Ertekin[7], Ayşe Boyvat[4], Atay Vural[8], Seçil Vural[7]
[1]Graduate School of Health Sciences, Koç University, Istanbul, Turkey
[2]Koç University Research Center for Translational Medicine, Koç University, Istanbul, Turkey
[3]Department of Surgery, Koç University School of Medicine, Istanbul, Turkey
[4]Department of Dermatology, Ankara University Faculty of Medicine, Ankara, Turkey
[5]Department of Dermatology, Başakşehir Çam ve Sakura Şehir Hastanesi, İstanbul, Turkey
[6]Department of Rheumatology, Koç University School of Medicine, Istanbul, Turkey
[7]Department of Dermatology, Koç University School of Medicine, Istanbul, Turkey
[8]Department of Neurology, Koç University School of Medicine, Istanbul, Turkey

Objective: To characterize the immune landscape of Behçet's Disease (BD) skin lesions through comprehensive single-cell and functional analyses, and to identify key cellular populations and molecular pathways involved in BD pathogenesis.
Materials-Methods: We performed single-cell RNA sequencing (scRNAseq), T cell receptor (TCR) sequencing, and flow cytometry on skin lesions from BD patients (n=22) and healthy controls (n=24). Additionally, matched peripheral blood mononuclear cells (PBMC) from BD patients (n=15) and healthy controls (n=15) were analyzed. Cells were isolated using a 3-hour enzymatic digestion protocol optimized for skin tissue. Cell type annotation was performed using the automated CellTypist algorithm. Differential abundance analysis was conducted using scCoda, and differential gene expression analysis was performed with Wilcoxon rank-sum test and Benjamini-Hochberg correction.
Results: scRNAseq and flow cytometry revealed significant enrichment of plasmacytoid dendritic cells (pDCs), mucosal-associated invariant T (MAIT) cells, natural killer (NK) cells, innate lymphoid cells type 3 (ILC3s), and memory B cells in BD skin lesions. BD lesional skin showed robust type I immune responses with heightened expression of cytotoxic molecules and increased production of IFN-γ and TNF-α by T helper cells, cytotoxic T cells, and MAIT cells. Pathway analysis demonstrated significant activation of the JAK-STAT signaling pathway. TCR sequencing revealed no increased T cell clonality in BD skin lesions, suggesting innate rather than adaptive T cell-driven inflammation.
Conclusions: Our findings reveal that innate immune cells and innate-like T lymphocytes, in conjunction with activated Th1/Tc1 pathways and JAK-STAT signaling, play central roles in BD skin pathogenesis. The absence of T cell clonality despite strong inflammatory responses highlights BD's primarily innate immune-driven nature. These results suggest potential therapeutic strategies targeting JAK-STAT pathway or type I/II interferon signaling in BD management.

Keywords: Behçet's disease, MAIT cells, NK cells, interferon, single-cell RNA sequencing

[OP-02]

B and T Lymphocyte Subgroups in Patients with Selective IgA Deficiency

Nilsu Izgi[1], Semra Demir[1], Nevzat Kahveci[3], Günnur Deniz[2]
[1]İstanbul University, Institute of Health Sciences, Istanbul, Turkiye
[2]Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Immunology, Istanbul, Turkiye
[3]Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine, Immunology and Allergy Diseases, Istanbul, Turkiye

Objective: Selective IgA deficiency is the most common congenital primary immunodeficiency, characterized by serum IgA concentrations below 7 mg/dL with normal IgG and IgM levels in patients over four years of age. Most primary immnodeficiencies arise from defects in B cells or impaired interactions between B and T cells. This study aims to analyze T cell subsets, T cell surface molecules, B cell subsets, and B cell surface molecules in patients with selective IgA deficiency and compare the findings with those in common variable immunodeficiency (CVID) patients.
Materials-Methods: Peripheral blood mononuclear cells were isolated from 14 selective IgA deficiency patients, 19 CVID patients and 11 healthy controls. Cells were labeled with anti-CD3, -CD4, -CD8, -CXCR5, -PD-1, -CD45RA, -CCR7, -CD25, CD127, -IgM, -IgD, -CD19, -CD21, -CD27 monoclonal antibodies. Following surface staining, the labeled cells were evaluated by flow cytometer.
Results: Patients with selective IgA deficiency had higher allergy rates, while CVID patients showed increased autoimmunity. Both groups had lower lymphocyte counts than controls. Selective IgA deficiency patients had higher CD4+ T cells, whereas CVID patients had an increase in CD8bright and CD8dim cells. T follicular cytotoxic cells were reduced in both groups. Switched memory B cells were lower in both conditions, while marginal zone-like B cells were decreased in selective IgA deficiency compared to CVID but increased in CVID compared to controls. Finally, CVID positively correlated with autoimmunity, suggesting its association with immune dysregulation.
Conclusions: Both selective IgA deficiency and CVID exhibit abnormalities in T and B cell differentiation, with notable similarities in lymphocyte subpopulations. However, these immune dysregulations are more pronounced in CVID, highlighting its greater impact on adaptive immunity.

Keywords: Selective IgA Deficiency, CVID, Adaptive Immune System, Allergy, Autoimmunity

[OP-03]

Asparagine availability controls germinal center B cell homeostasis

Yavuz F. Yazicioglu[1], Eros Marin[1], Hana Andrew[1], Karolina Bentkowska[1], Julia Johnstone[1], Robert Mitchell[1], Zhi Wong[1], Kristina Zec[1], Joannah Fergusson[1], Mohammad Ali[2], James Macrae[3], Alexander J. Clarke[1]
[1]University of Oxford / Kennedy Institute of Rheumatology, NDORMS, Oxford, United Kingdom
[2]University of Oxford / Mahidol Oxford Tropical Medicine Research Unit, Oxford, United Kingdom
[3]The Francis Crick Institute, London, United Kingdom

The germinal center (GC) reaction is crucial for humoral immunity, driving antibody affinity maturation through iterative cycles of B cell selection and mutational enrichment of their receptors. GC B cells proliferate at some of the highest rates among mammalian cells, yet the metabolic mechanisms that enable this are poorly defined. Using integrated metabolomic and transcriptomic profiling, we found that metabolism of the non-essential amino acid asparagine (Asn) was highly upregulated in GC B cells compared to their naive counterparts. Asn was conditionally essential to B cells, and its biosynthetic enzyme, asparagine synthetase (ASNS), was upregulated following activation, most notably in the absence of Asn, through the integrated stress response sensor general control non-derepressible 2 (GCN2). Conditional deletion of *Asns* in B cells reduced survival and proliferation in low Asn conditions. Removal of environmental Asn by its degradative enzyme asparaginase or dietary restriction compromised the GC reaction, impairing affinity maturation and the humoral response to influenza infection *in vivo*. Stable isotope tracing and single-cell RNA sequencing revealed that metabolic adaptation to the absence of Asn required ASNS and that oxidative phosphorylation and nucleotide synthesis were particularly sensitive to Asn deprivation, which could be rescued by exogenous nucleoside supplementation. Altogether, our findings establish Asn metabolism as a critical regulator of GC B cell homeostasis and highlight its potential to control immune responses in health and disease.

Keywords: Germinal center, asparagine, immunometabolism, B cells

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[OP-04]

Phosphorylation of signaling molecules in peripheral and thymic B cells and responses to T follicular helper cell-related cytokines in myasthenia gravis

Cemre Aktaş[1], Arman Çakar[2], Fikret Aysal[5], Hacer Durmuş[2], Ali Altay[3], Salih Duman[4], Gülçin Yeğen[3], Berker Özkan[4], Yeşim Parman[2], Güher Saruhan Direskeneli[1]
[1]Department of Physiology, Istanbul Medical Faculty, Istanbul University; Department of Molecular Biology & Genetics, Istanbul Technical University, Istanbul, Turkey
[2]Department of Neurology, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey
[3]Department of Pathology, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey
[4]Department of Thoracic Surgery, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey
[5]Private Practice, Istanbul, Turkey

INTRODUCTION
Myasthenia gravis (MG) is an autoimmune disorder characterized by autoantibody production. Responses of autoreactive B cell populations to T follicular helper (Tfh) cell-related cytokines may contribute to the disease pathogenesis.

OBJECTIVE
To study spontaneous B cell responses and alterations to cytokine stimulations in MG patients compared to healthy controls (HCs).

METHODS
A total of 23 acetylcholine receptor (AChR) antibody positive MG patients and 21 HCs were recruited. Nine patients were on immunosuppressive treatment. Peripheral blood mononuclear cells (PBMCs) and thymocytes (n=5) were isolated and stained for CD19, CD20, CD27 and IgD. Furthermore, cells were stimulated *in vitro* with IFNα2a, IFNβ, IL-21 and IL-4. B cell phenotypes and phosphorylation of STAT proteins (pSTAT-1, -3 and -6) in $CD20^+$ B cells were compared.

RESULTS
Ex vivo without cytokine stimulation, pSTAT-1 (both T701 and S727) and pSTAT-3 (both T705 and S727) were significantly higher in $CD20^+$ B cells of MG patients compared to HCs, while pSTAT-6 (Y641) tended to be increased in MG compared to HCs. When the cells were stimulated with Tfh-related cytokines, attenuated phosphorylation of pSTAT-3 (both T705 and S727) in response to IL-21 and pSTAT-6 (Y641) to IL-4 were observed in $CD20^+$ B cells of MG. Both IFNα2a and IFNβ induced no significant changes in pSTAT-1 (both T701 and S727) compared to HCs. Phenotypic analysis of these MG patients showed significantly lower $CD27^+IgD^+$ un-switched memory and $CD27^+IgD^-$ switched memory B cells compared to HCs, while $CD27^-IgD^+$ naïve as well as $CD27^-IgD^-$ B cells were higher in MG. Isolated thymocytes with or without cytokine stimulation revealed lower levels of STAT phosphorylation in thymic B cells than the peripheral blood.

CONCLUSIONS
MG patients exhibit altered phosphorylation profiles of pSTAT proteins in B cells, characterized by elevated basal phosphorylation, suggesting a sustained IL-21 and possibly IFN type I response in disease pathogenesis.

Keywords: *autoimmunity, cytokines, B cells, STATs, phosphorylation*

[OP-05]

Enhancing the Breadth of Protection from *Neisseria Meningitidis* Infection via Bivalent OMV Vaccine

Zeynep Arzum[1], Tugce Canavar Yıldırım[6], Yasemin Ozsurekci[4], Muzaffer Yildirim[2], Irem Evcili[2], Volkan Yazar[3], Kubra Aykac[4], Ulku Guler[5], Bekir Salih[5], Mayda Gursel[2], Ihsan Gursel[2]
[1]Izmir Biomedicine and Genome Center, Izmir, Turkiye; Department of Genome Sciences and Molecular Biotechnology, Izmir International Biomedicine and Genome Institute, Dokuz Eylul University, Izmir, Turkiye
[2]Izmir Biomedicine and Genome Center, Izmir, Turkiye
[3]Department of Molecular Biology and Genetics, Bilkent University, Ankara, Turkiye
[4]Division of Pediatric Infectious Diseases, Department of Pediatrics, Faculty of Medicine, Hacettepe University, Ankara, Turkiye
[5]Department of Chemistry, Hacettepe University, Ankara, Turkiye
[6]Izmir Biomedicine and Genome Center, Izmir, Turkiye; Department of Molecular Biology and Genetics, Bilkent University, Ankara, Turkiye

The major serogroups of *Neisseria meningitidis* (A, B, C, W, and Y) cause invasive meningococcal disease. Currently, no single vaccine targets both serogroups ACWY and B simultaneously. In Türkiye, *N. meningitidis* serogroups B (MenB) and W (MenW) are the most common causes of meningococcal infections. To address this, we developed a CpG ODN-adjuvanted, alum-adsorbed, bivalent outer membrane vesicle (OMV)-based vaccine designed to provide broad protective coverage with lesser doses.
OMVs were purified from MenW and MenB serogroups using a HiScreen Capto Core 400 column on an ÄKTA-GO fast protein liquid chromatography system. The OMVs were then formulated with single or dual adjuvants or their combinations. The immunogenicity of the bivalent MenW+MenB OMV vaccine was evaluated in a murine model and compared with two commercially available meningococcal vaccines. BALB/c mice were immunized at two-week intervals with the candidate vaccine, while control groups received either a licensed MenACYW conjugate vaccine or a MenB OMV-based vaccine (4CMenB). Immune responses were assessed either by measuring antibody titers via IgG ELISA or via serum bactericidal activity (SBA) assays.
The MenW+MenB OMV vaccine induced significantly higher antibody responses and SBA titers than the control groups. Moreover, SBA titers were comparable to those elicited by the licensed 4CMenB vaccine and higher than those induced by the MenACYW conjugate vaccine (analyzed using one-way ANOVA with Dunnett's multiple comparisons test). Moreover, unrelated MenX specific SBA activity of the bivalent vaccine was found to be 10^3 log higher than the licenced 4CmenB vaccine. These findings highlight the vaccine's strong immunogenicity and support its potential to address a critical gap in meningococcal disease prevention, particularly in regions where diverse serogroups are prevalent. The MenW+MenB OMV vaccine could serve as an effective alternative or complementary option in meningococcal immunization strategies.

Keywords: Meningococcus, vaccine, *N. meningitidis*, OMV, CpG ODN adjuvant

[OP-06]

Discovery of Tumor-Specific T Cell Receptors Through Single-Cell Sequencing of Tumor-Infiltrating Lymphocytes in Melanoma

Elif Çelik[1], Betül Çakıcı[2], Beyza Nur Uysak[2], Mehmet Karaçay[3], Batuhan Yağcıoğlu[4], Serra Özarı[5], Serkan Yazıcı[6], Süleyman Çeçen[7], Diğdem Yöyen Ermiş[4], Haluk Barbaros Oral[4], Tolga Sütlü[8]
[1]Acıbadem Mehmet Ali Aydınlar University, Graduate School of Health Sciences, Department of Translational Medicine;Acıbadem Mehmet Ali Aydınlar University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics
[2]Acıbadem Mehmet Ali Aydınlar University, Graduate School of Natural and Applied Sciences, Department of Molecular Biology and Genetics;Acıbadem Mehmet Ali Aydınlar University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics
[3]Van Yüzüncü Yıl University, Faculty of Medicine, Department of Internal Medicine, Division of Medical Genetics
[4]Bursa Uludağ University, Faculty of Medicine, Department of Immunology
[5]Boğaziçi University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics;Acıbadem Mehmet Ali Aydınlar University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics
[6]Bursa Uludağ University, Faculty of Medicine, Department of Dermatology and Venereal Diseases
[7]Bursa Uludağ University, Faculty of Medicine, Department of Plastic, Reconstructive and Aesthetic Surgery
[8]Acıbadem Mehmet Ali Aydınlar University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics

Objective: T cell receptor (TCR)-based immunotherapies have emerged as a promising strategy for cancer treatment, particularly in melanoma, where tumor-infiltrating lymphocytes (TILs) play a crucial role in antitumor immunity. TIL therapy has recently received FDA approval for treating advanced melanoma; however, its clinical application remains practically challenging.
Our study aims to identify tumor-specific TCRs by performing single-cell RNA (scRNA-seq) and TCR sequencing on melanoma-derived TILs. By characterizing TCR repertoires and their antigen specificity, we seek to develop a framework for enhancing personalized TCR-based therapies, including TCR-NK cell therapies, to improve immunotherapy in melanoma.
Materials-Methods: Melanoma tumor samples were obtained from eleven patients undergoing surgical resection. TILs from two patients were isolated and expanded for scRNA-seq and paired TCRα/β sequencing to identify clonally expanded T cells with tumor-reactive properties. Additionally, $CD8^+$ T cells isolated from peripheral blood were co-cultured with tumor cells to grow tumor-reactive clones and subjected to scRNA-seq. Computational analysis was performed to classify TCR clonotypes based on frequency and their potential tumor reactivity.
Results: scRNA-seq revealed a clonally expanded TCR repertoire with potential effector signatures. For the dominant clonotypes, a detailed analysis of TCR VDJ sequences was carried out and paired TCRα/β sequences including CDR and constant region sequences were extracted. A 3rd generation lentiviral vector was designed for the expression of novel TCRs, incorporating a 2A-peptide based design for the paired expression of TCRα/β sequences.
Conclusion: Our study successfully identified tumor-specific TCRs from melanoma-derived TILs and peripheral CD8+ T cells using scRNAseq. Functional validation of discovered sequences using TCR-NK technology is ongoing. Our approach seems to be feasible in establishing a functional pipeline for discovery of novel tumor-specific TCRs. These findings provide a foundation for TCR-based immunotherapies, potentially leading to novel personalized treatments such as TCR-NK for melanoma patients.

Acknowledgement:This Project supported by the TUBITAK 1003 program (Projet No: 218S897).

Keywords: Tumor-Infiltrating Lymphocytes (TILs), T Cell Receptor (TCR), Single-Cell Sequencing, TCR-NK Cells

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[OP-07]

End-to-end Vector and Process Development for Clinically Applicable CD19-targeted CAR-T Cell Production in Türkiye

Elif Çelik[1], Betül Çakıcı[2], Mertkaya Aras[3], Beyza Nur Uysak[2], Serra Özarı[4], Esra Erdal[5], Batu Erman[6], Tolga Sütlü[6]
[1]Acıbadem Mehmet Ali Aydınlar University, Graduate School of Health Sciences, Department of Translational Medicine;Acıbadem Mehmet Ali Aydınlar University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics, İstanbul, Türkiye
[2]Acıbadem Mehmet Ali Aydınlar University, Graduate School of Natural and Applied Sciences, Department of Molecular Biology and Genetics;Acıbadem Mehmet Ali Aydınlar University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics, İstanbul, Türkiye
[3]Sabancı University, Faculty of Engineering and Natural Sciences, Molecular Biology, Genetics and Bioengineering Program, İstanbul, Türkiye
[4]Boğaziçi University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics;Acıbadem Mehmet Ali Aydınlar University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics, İstanbul, Türkiye
[5]Izmir Biomedicine and Genome Center, İzmir, Türkiye
[6]Acıbadem Mehmet Ali Aydınlar University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics, İstanbul, Türkiye

Objective: Chimeric Antigen Receptor (CAR) T cell therapy has revolutionized cancer immunotherapy by enabling autologous T cells to selectively target tumor antigens such as CD19 in B-cell malignancies. This study aims to establish an end-to-end vector and process development pipeline for the clinical-grade production of CD19-targeted CAR-T cells in Türkiye.Our work focuses on optimizing lentiviral vector design and T cell expansion strategies to enhance genetic modification efficiency as well as functional persistence, cytotoxicity, and safety profiles. Additionally, we seek to develop a GMP-compliant, scalable, and cost-effective manufacturing process that aligns with global regulatory standards.

Materials-Methods: A clinically applicable lentiviral backbone, pANTER23, was designed. This vector incorporates a CAR construct derived from the FMC63 antibody and includes CD28 and CD3ζ costimulatory domains. Activation assays were performed using Jurkat cells to evaluate CAR expression and functionality. Optimization of 3rd generation lentiviral vector production was conducted using 293FT cells to enhance viral yield. Activation, expansion and transduction conditions for primary T cells were optimized using PBMC samples from healthy donors. Furthermore, the optimized process was tested in vitro using T cells isolated from patients with B-CLL and functional assays were performed for evaluation of CAR-T cell activity.

Results: Our results indicate that patient derived, pANTER23-FMC63 CAR-T cells show excellent fold expansion (over 300 fold in 14 days) and a transduction efficiency of over %30. CAR-T cells prepared according to our optimized protocol demonstrate high cytotoxicity and cytokine secretion against CD19+ cell lines and most importantly against autologous tumor cells.

Conclusion: Our findings suggest that pANTER23-FMC63 CAR-T cells exhibit robust in vitro expansion, efficient transduction, and potent activity against CD19+ tumor targets. These results highlight the potential of this optimized platform for developing clinically applicable CAR-T cell therapies in Türkiye and improving accessibility of cellular immunotherapies in the region.
Acknowledgement:This Project is supported by TUBITAK 1004 program(No: 20AG006).

Keywords: Chimeric Antigen Receptor, T cells, Lentiviral Vector

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[OP-08]

Therapeutic Use of Bone Marrow-Derived Tolerogenic Dendritic Cells Modified by Lentiviral Transduction in CIA Model

Gözde Arslan[1], Fatmanur Dündar[1], Elif Çelik[2], Ezgi Yumuşak[3], Diğdem Yöyen Ermiş[4], Tolga Sütlü[2], Ahmet Akkoç[3], Murat Yalçın[5], Haluk Barbaros Oral[4]
[1]Department of Medical-Immunology, Institute of Health Science, Bursa Uludag University, Bursa, Türkiye.-Department of Immunology, Faculty of Medicine, Bursa Uludag University, Bursa, Türkiye.- Department of Medicine, Laboratory Animal Breeding and Research Unit (DENHAB), Bursa Uludağ University,16059,Bursa, Türkiye.
[2]Acıbadem Mehmet Ali Aydınlar Unıversity, Graduate Scholl of Health Sciences,Department of Translational Medicine Istanbul, Türkiye
[3]Department of Pathology, Institute of Health Science, Bursa Uludag University Faculty of Veterinary Medicine, Bursa, Türkiye
[4]Department of Immunology, Faculty of Medicine, Bursa Uludag University, Bursa, Türkiye.-Department of Medicine, Laboratory Animal Breeding and Research Unit (DENHAB), Bursa Uludağ University,16059, Bursa, Türkiye.
[5]Department of Physiology, Institute of Health Science, Bursa Uludag University Faculty of Veterinary Medicine, Bursa, Türkiye- Department of Medicine, Laboratory Animal Breeding and Research Unit (DENHAB), Bursa Uludağ University,16059, Bursa, Türkiye.

Introduction: Rheumatoid arthritis-(RA) is a chronic autoimmune disease that affects approximately 1% of the population, leading to progressive joint damage and disability. Although its exact cause remains unclear, genetic predisposition-(HLA-DRB1) and environmental factors such as smoking contribute to its development.RA is characterized by chronic synovial inflammation, primarily in small joints, and, if untreated, can cause irreversible joint damage. While NSAIDs-DMARDs, and biologics help manage symptoms, a definitive cure is lacking, and treatment responses vary among patients.

Method: In the first strategy, DCs were modified with a lentiviral vector encoding only the expression of IDO, which catabolizes tryptophan. In the second approach, DCs were also modified with a lentiviral vector encoding a fusion protein called CTLA4-KDEL. In the third approach, DCs were transduced with a lentiviral vector encoding both IDO overexpression and shRNA-mediated silencing of CD80/CD86 within the same vector construct. Genetically modified DCs were administered intra-articularly to collagen-induced-RA model mice. Treated RA model were followed for two weeks. Finally, histopathological examination was performed to evaluate joint inflammation and tissue damage in the affected joints.

Result and Conclusion: After transduction into DCs, ELISA analysis confirmed upregulation of IDO in the group expressing the IDO construct and IDO overexpression and shRNA-mediated silencing of CD80/CD86 within the same vector construct. Flow cytometry showed suppression of surface markers CD80 and CD86 as a result of CTLA4 transduction, indicating a tolerogenic phenotype. We demonstrated a significant decrease in the clinical scores of CIA model animals after injection of DHs, which were given tolerogenic properties using three different strategies. Tolerance-inducing DCs have exciting potential for the treatment of RA and other autoimmune diseases.

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Keywords: Rheumatoid Arthritis, Dendritic Cells, indoleamine-2, 3-dioxygenase, Lentiviral Transduction, CD80/86

[OP-09]

Investigating the tumor immune microenvironment through innovative data analysis interfaces

H Atakan Ekiz
Department of Molecular Biology and Genetics, Izmir Institute of Technology, Izmir, Turkey

Objective: The Cancer Genome Atlas (TCGA) provides vast gene expression datasets across cancer types, but their analysis often requires advanced coding skills, limiting accessibility. Existing tools allow data exploration but lack integrated platforms for in-depth analyses of the tumor microenvironment (TME) without coding expertise. To bridge this gap and enhance accessibility, we developed The Cancer Genome Explorer (TCGEx) - a user-friendly, web-based platform enabling comprehensive analyses of cancer gene expression data.

Methods: TCGEx, built using the R/Shiny framework, integrates preprocessed TCGA and immune checkpoint inhibition (ICI) study datasets. The platform offers ten analysis modules, including survival modeling, gene set enrichment analysis, principal component analysis, correlation analysis, hierarchical clustering, and machine learning (ML)-based feature selection. TCGEx enables users to upload and analyze their own datasets, leveraging its advanced analysis pipelines across diverse research contexts.

Results: Using TGCEx, we studies gene expression patterns associated with immunity and immunotherapy response in TCGA skin cutaneous melanoma (SKCM). We set out to identify microRNAs associated with intratumoral interferon-gamma signaling, as it remains one of the best prognosticators in cancer. Lasso machine learning analysis of SKCM and external melanoma datasets identified a set of microRNAs linked to IFNγ response, with miR-155 emerging among the strongest predictors. Examination of existing scRNAseq data indicated heterogeneous expression patterns of these miRNAs within the TME suggesting functional involvement in different cell types. Notably, while higher baseline miR-155 expression correlated with improved overall survival in melanoma, it was not a strong standalone predictor of the ICI response. Finally, we broadened our search for response predictors to include chemokine and chemokine receptor genes and identified a five-gene signature strongly associated with improved ICI response across multiple cancer types and treatment regimens.

Conclusion: TCGEx emerges as a powerful and accessible tool for analyzing high throughput data in cancer leading to novel insights.

Keywords: transcriptomics, immunotherapy, machine learning, microRNAs, visualization

[OP-10]

Investigation of SARS-CoV-2 Nucleocapsid and Spike N501Y Variant-Specific IgG Subtypes in Convalescent Pediatric Patients from COVID-19 Using Multiplex Flow Cytometric Bead-Based Assay

Mehmet Karacay[1], Mehmet Karacay[2], Zeynep Ece Yildiz[3], Fatma Oyku Elaslan[3], Mensure Feray Cosar[1], Muhammed Yuksel[4], Cigdem Arikan[4], Dilek Guller[5], Nevin Hatipoglu[6], Ayca Sayı Yazgan[1]
[1]Department of Molecular Biology and Genetics, Faculty of Science and Letters, Istanbul Technical University, 34469 Istanbul, TÜRKİYE.
[2]Department of Medical Genetics,Faculty of Medicine, Van Yuzuncu Yıl University, 65080 Van, TÜRKİYE.
[3]Graduate School of Molecular Biology-Genetics and Biotechnology Istanbul Technical University, 34469 Istanbul, TÜRKİYE.
[4]Koç University School of Medicine, Department of Pediatric Gastroenterology and Hepatology, Koç University Translational Medicine Application and Research Center (KUTTAM), Istanbul, TÜRKİYE
[5]Demiroğlu Science University, Florence Nightingale Hospital, Department of Pediatric Gastroenterology, Istanbul, TÜRKİYE
[6]Bakırköy Dr. Sadi Konuk Training and Research Hospital, Pediatric Infectious Diseases, Istanbul, TÜRKİYE

Objective:
Compared to adults, children have a less comprehensive and efficient immunological response since their immune systems are still developing. This condition leads to a less severe progression of the disease for pediatric patients (1). Consequently, comprehending the immune response mechanisms in pediatric SARS-CoV-2 patients is crucial (2). Using the multiplex flow cytometry bead-based assay we aim to determine the time-dependent (1/3/6/9/12 months) changes in antibody levels of IgG and IgG subclasses (IgG1, IgG2, IgG3, and IgG4) produced against the Spike N501Y variant and the SARS-CoV-2 Nucleocapsid (NCP) in pediatric patients who had recovered from COVID-19.

Materials-Methods:
A total of n=84 pediatric patients aged 18 months to 18 years, who had recovered from COVID-19, were included in the study. Plasma samples were collected a minimum of 25 days after PCR positivity, and the levels of IgG and IgG subclasses specific to the NCP and Spike-N501Y variants in plasma were measured using flow cytometry. The data were analyzed using FlowJo software, and statistical significance was assessed using GraphPad Prism 9.

Results and Discussion:
A significant increase in the NCP-specific IgG3 subclass was observed between 9 and 12 months. A notable elevation in IgG4 levels was seen from 1 to 12 months. Regarding the Spike N501Y variant, the IgG3 subclass was predominant but showed a time-dependent decline from 6th month onward. In patients who experienced severe disease, NCP-specific IgG4 antibody levels significantly increased compared to 1 st month, while N501Y-specific IgG1 and IgG3 responses were dominant and consistent with the literature. Furthermore, in children with severe disease compared to mild cases, IgG4 responses at 9 and 12 months were significantly elevated compared to the 1st month, suggesting a contribution to immune homeostasis.

This study is supported by The Scientific and Technological Research Council of Türkiye (TUBITAK 1001), Project No:120S804

Keywords: SARS-COV-2 Specific Antibody, COVID-19, Cytometric Multiplex Bead Assay

[OP-11]

Production of nanobodies targeting IL-1R1 (interleukin-1 receptor type 1)

Gizem Çile[1], Alp Ertunga Eyüboğlu[3], Ahmet Selim Üzüm[3], Batu Erman[2], Sema Sırma Ekmekçi[4], Ahmet Gül[5]
[1]Acibadem University, Graduate School of Health Sciences, Istanbul, Turkey
[2]Acibadem University, Department of Molecular Biology and Genetics, Istanbul, Turkey
[3]Bogazici University, Department of Molecular biology and Genetics, Istanbul, Turkey
[4]Istanbul University, Aziz Sancar Experimental Medicine Research Institute, Istanbul, Turkey
[5]Istanbul University, Department of Internal Medicine Sciences, Istanbul, Turkey

IL-1R1 is an innate immune receptor that is involved in the regulation of inflammation. Defects in the IL-1R1 pathway can lead to inflammatory diseases. Currently, competitive inhibitors of IL-1R1, such as Anakinra (Kineret), are used to target IL-1R1. Nanobodies are recombinant proteins derived from the variable fragments of heavy-chain-only antibodies (VHHs). They are attractive candidates as anti-inflammatory therapeutics due to their small size, high stability and high solubility. Therefore, in this project, we aimed to produce nanobodies against IL-1R1 to develop alternative treatments for IL-1R1 mediated inflammatory diseases.

Nanobodies derived from llamas immunized with human IL-1R1 (hIL-1R1) were generated by B lymphocyte VHH library screening followed by phage display. A total of 136 nanobody clones were identified by their ability to bind hIL-1R1. These nanobodies were expressed in bacteria and purified using Fast Protein Liquid Chromatography (FPLC). Surface Plasmon Resonance (SPR) technology was used to characterize the binding affinities of these nanobodies to the hIL-1R1. Additionally, epitope binning experiments were performed to assess whether the high affinity nanobodies could block each other's binding to the receptor. Finally, the inhibitory activities of these high affinity nanobodies were analyzed in cell culture experiments.

The equilibrium dissociation constant (KD) is inversely correlated with binding affinity. Because Anakinra has a KD of approximately 50 pM and IL-1b has a KD value around 2 nM, nanobodies with KD values between 1 pM and 1 nM were classified as high affinity nanobodies. Out of 136 nanobodies, more than ten exhibited high affinity for hIL-1R1. Among them, two nanobodies successfully inhibited hIL-1R1 signaling in cell culture. These nanobodies are promising candidates as anti-inflammatory biologicals. However, their in vivo efficacy still needs to be investigated. Epitope binning experiments revealed that their binding was not affected by other nanobodies, suggesting their potential for the development of multivalent nanobody therapeutics.

Keywords: Nanobody, interleukin-1 receptor type 1, inflammatory diseases

[OP-12]

Investigating alternative polyadenylation sites in tumor-associated macrophage differentiation at single-cell resolution

Ebru Kocakaya[1], Hamdullah Yanık[2], Hilal Kazan[3], Ayşe Caner[4], Güneş Esendağlı[2], Yasin Kaymaz[5]
[1]Ege University, Graduate School of Natural and Applied Sciences, Department of Biotechnology, İzmir,Türkiye
[2]Hacettepe University, Cancer Institute, Department of Basic Oncology, Ankara Türkiye
[3]Antalya Bilim University, Department of Computer Engineering, Antalya, Türkiye
[4]Ege University, Department of Basic Oncology, İzmir, Türkiye
[5]Ege University, Faculty of Engineering, Bioengineering Department, İzmir, Türkiye

Objective: Lung cancer remains a leading cause of cancer-related mortality worldwide, with the tumor microenvironment (TME) playing a critical role in disease progression. Macrophages, key immune components of the TME, exhibit significant plasticity, differentiating into pro-inflammatory M1 macrophages with anti-tumor effects or immunosuppressive M2 macrophages that promote tumor progression and correlate with poor prognosis. However, distinguishing between these subtypes remains challenging. In this study, we utilized 3' single-cell RNA sequencing (scRNA-Seq) to investigate alternative polyadenylation (APA) profiles in macrophage polarization under lung cancer tumor microenvironment mimicking conditions and normal conditions. Additionally, we analyzed APA profiles of tumor samples across various lung cancer stages.
Materials-Methods: Human PBMC-derived monocytes were polarized into M1 and M2 macrophages, with the experimental group exposed to A549 lung cancer cell-conditioned media to mimic the TME. Using the well-based Seq-Well method, we generated transcriptomic data from 10,807 single cells across 11 treatment conditions and time points. APA events were analyzed with our tool, PeakATail, constructing a cell-by-PAS (polyadenylation site) matrix to enhance macrophage classification. Additionally, we used **PeakATail** to analyze a publicly available scRNA-Seq lung cancer dataset (GSE123902), which includes primary tumors (Stages I, II, and IV), metastases (bone, brain, and adrenal), and tumor-adjacent tissues.
Results: APA isoforms of *NEK1, SLC39A10, POLA1, THADA, and WDPCP* emerged as potential macrophage differentiation markers. Furthermore, APA signatures were associated with tumor stages and metastatic profiles.
Conclusion: Our findings suggest that APA profiling offers a novel approach for classifying tumor-associated macrophages (TAMs). Future research incorporating APA-based insights into machine learning models could refine macrophage subtype classification and deepen our understanding of macrophage plasticity in cancer.

Keywords: macrophage differentiation, alternative polyadenylation sites, single-cell RNA-Seq, lung cancer

[OP-13]

Promoting improved agnostic cross presentation of tumour antigens with an oncolytic adenovirus expressing bispecific macrophage engagers

Ahmet Hazini, Len Seymour
Department of Oncology, University of Oxford, Oxford, UK

Non-specific ('agnostic') cross-presentation of tumour antigens (TAA) is an essential process for generating efficient adaptive immune responses against cancer. APCs can uptake TAA by phagocytosis of proteins from cancer cells, and then present the antigens on HLA molecules to T cells to activate them. To induce cancer cell uptake by the APCs, we aimed develop a bispecific macrophage engager (BiME) that can bring cancer cells and APCs into close proximity and we hypothesised that oncolytic viruses could be used to express BiMEs in the tumour microenvironment.

We generated an oncolytic adenovirus expressing BiME and showed that expressed bispecific protein can efficiently bind to ligands on the surface of APCs and cancer cells. Co-culture assays were conducted to evaluate phagocytosis, macrophage activation, and antigen cross-presentation. To analyse antigen cross-presentation, we engineered NY-ESO-1 expressing human HLA-A2 negative cancer cells and co-cultured them with HLA-mismatched HLA-A2 positive APCs. NY-ESO-1(157-165) peptide loaded HLA-A2 specific 1G4 TCR primary T cells were used to detect cross-presentation. Finally, we utilised freshly dissected colorectal tumour and normal colon biopsy samples to assess cancer-specific virus replication, BiME generation, and macrophage activation.

The BiME significantly enhanced the phagocytosis of cancer cells by macrophages, surpassing the efficacy of the clinically tested anti-CD47 monoclonal antibody. The BiME further activated macrophages, leading to the upregulation of CD86, CD80, and HLA-DR expression. Notably, the combination of oncolytic virus and BiME resulted in the strongest macrophage activation. Tumour antigen cross-presentation by macrophages was markedly increased following BiME treatment. Ex vivo primary tissue experiments demonstrated that oncolytic viruses replicated and produced BiME exclusively in tumour biopsy samples, where they also induced macrophage activation, unlike in normal tissue samples.

Taken together, we show that an oncolytic adenovirus encoding BiMEs provides a promising new mechanism for antigen-agnostic cross presentation in the development of cancer vaccines for solid tumours.

Keywords: cancer vaccines, immunotherapy, oncolytic viruses

[OP-14]

Leishmania infantum Extracellular Vesicles Mediate Protection from Visceral Leishmaniasis and Long-Term Cross Protection from Cutaneous Leishmaniasis

Ismail Cem Yilmaz[1], Ibrahim Tokmak[2], Muzaffer Yıldırım[1], Elif Sena Özkoç[1], Irem Evcili[1], Bilgi Güngör[1], Volkan Yazar[3], Bekir Salih[4], Ülkü Güler[4], Ihsan Gürsel[1], Mayda Gürsel[1]
[1]Izmir Biomedicine and Genome Center, Izmir, TURKIYE
[2]Dokuz Eylul University, Izmir International Biomedicine and Genome Institute, Department of Molecular Biology and Genetics, Izmir, TURKIYE
[3]Bilkent University, Faculty of Science, Department of Molecular Biology and Genetics, Ankara, TURKIYE
[4]Hacettepe University, Faculty of Science, Department of Chemistry, Ankara, TURKIYE

Introduction
Leishmaniasis is a vector-borne disease caused by various Leishmania protozoan parasites. Depending on the infecting species and host factors, the disease manifests as cutaneous, mucocutaneous, or visceral leishmaniasis. As a neglected tropical disease, leishmaniasis poses a significant global health problem, necessitating the urgent need for an effective broad vaccine to control its spread.
Objectives & Methods
Here, we investigated the immunoprotective efficacy of Leishmania infantum-derived extracellular vesicles (Li-EVs) in a murine model of L. infantum-generated visceral leishmaniasis, and L. major-generated cutaneous leishmaniasis. Li-EVs were isolated and purified from axenically cultured metacyclic promastigotes under GMP-adaptable conditions and subsequently characterized. 6–8-week-old BALB/c mice were immunized with Li-EVs, and the humoral immune response was evaluated by measuring soluble Leishmania antigen (SLA)-specific antibody levels using ELISA at two weeks and four months post-booster immunization. To assess the potential cis and cross-protection augmented by Li-EVs, mice were challenged with live luciferase-expressing transgenic L. infantum and L. major parasites, two weeks and four months post-vaccination, respectively. Parasite loads were monitored using in vivo imaging system (IVIS), longitudinally.
Results & Conclusion
Li-EV-immunized groups demonstrated significantly higher both L. major and L. infantum SLA-specific antibody titers compared to the placebo group. Further, Li-EV-immunized mice demonstrated ~2,5 fold lower parasite loads significantly post-challenge with L. infantum, and ~4,5 fold lower parasite loads significantly post challenge with L. major. These findings indicate that Li-EVs harbor shared immunogenic antigens with L. major, which can induce sustained and cross reactive protective immune responses, highlighting their potential as a standalone candidate vaccine against leishmaniasis.
Acknowledgement
This work is partially supported by TUBITAK, 1004-22AG013 and 1003-115S073 grants awarded to IG and MG respectively.

Keywords: L. infantum, Extracellular vesicles, vaccine, IVIS

[OP-15]

Immunological Profiling Following SARS-CoV-2 Vaccination

Irem Evcili, Hatice Asena Sanli, Ihsan Gursel, Mayda Gursel
Izmir Biomedicine and Genome Center, Izmir, Turkey

Virus-like particles (VLPs) are recognized as a promising vaccine platform due to their capacity to elicit robust immunogenic responses. Their structural characteristics facilitate effective antigen presentation and cross-linking of B cell receptors, leading to robust antigen-specific humoral immunity. In this study, we aimed to evaluate the immunogenicity of a VLP vaccine encoding the four structural proteins (Spike, Nucleocapsid, Envelope, Membrane) of the SARS-CoV-2 virus. Specifically, we determined the effective dose (ED50) of the vaccine, optimized adjuvant concentrations (Alum/CpG ODN) and assessed age- and sex-dependent immune responses elicited by the vaccine in preclinical mouse models. For this, BALB/c and C57BL/6 mice were immunized subcutaneously with varying doses of VLP adjuvanted with different concentrations of Alum and CpG ODN. Humoral responses against Spike, RBD and Nucleocapsid were quantified using ELISA, while cellular immunity was assessed by cytokine profiling using cytometric bead arrays after ex-vivo antigen recall of splenocytes. The VLP vaccine exhibited dose-dependent immunogenicity, with an ED50 of 2.83 µg. Optimal adjuvant combinations of 120 µg Alum + 60 µg CpG ODN elicited robust Th1-skewed responses. As expected, older mice displayed decreased anti-S IgG titers compared to younger cohorts. Sex-based differences were more pronounced in adult mice than young and older cohorts, with females generally exhibiting higher anti-S and anti-N IgG titers than males. Interestingly, RBD-specific responses were higher than females in adult mice. In summary, our results indicate that the VLP vaccine platform can elicit strong Th1-polarized immunity, dose-dependent efficacy, and adaptability across different age groups and sexes.

Keywords: Virus-like particle (VLP), SARS-CoV-2, Vaccine

[OP-16]

Development and Evaluation of the Efficacy of a Novel Monoclonal Antibody Targeting CX3CR1 for Pain Relief in Pancreatic Cancer, Chronic and Acute Pancreatitis

Deniz Baybağ[1], Tayyar Emir Gözeler[4], Nedim Can Çevik[1], Başak Kavaklıoğlu[1], Sıla Sığırlı[1], Didem Zeybek[1], Ihsan Ekin Demir[3], Güralp Onur Ceyhan[2]
[1]medical biotechnology, Acibadem university,istanbul, turkey
[2]General surgery, Acibadem university,istanbul, turkey
[3]Department of surgery, Technical university of munich, munich, germany
[4]Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Acibadem Mehmet Ali Aydınlar University, Istanbul, Turkey

Introduciton
Pancreatic cancer, chronic and acute pancreatitis present daunting challenges due to the presence of severe pain and unfortunately the absence of effective treatments. Nervous system is activated within the tissue, and there is an increase in the expression and activity of the fractalkine chemokine and its specific receptor, CX3CR1, in parallel with the neuron-immune cell interaction in these diseases. Therefore, targeting this receptor holds promise for pain relief in afflicted patients.
Methods
Through hybridoma technology, a mAb was produced against the third terminal loop of CX3CR1 receptor. Supernatants collected from selected clones were purified. The optimal dosage was determined by SRB assay. Changes in pain, the histological and immunological progression of the diseases were evaluated on chronic and acute pancreatitis mouse models. The effects of the produced mAb will then be examined on "KPC" mouse model.
Results
Our newly developed monoclonal antibody exhibited 15% greater inhibition of pancreatic cancer cell proliferation compared to a commercially available antibody, while showing no cytotoxicity toward healthy pancreatic cells. In acute and chronic pancreatitis models, treatment with our antibody led to a 75% reduction in abdominal pain in chronic pancreatitis and a 51% reduction in acute pancreatitis, as assessed by the von Frey pain test. Additionally, treated mice exhibited a significant decrease in amylase and lipase levels, indicating improved pancreatic function. Histological analysis revealed reduced fibrosis and better preservation of pancreatic tissue integrity compared to untreated controls.
Conclusion
Due to the extremely high cost of existing inhibitory antibodies and the high renal toxicity of small molecule inhibitors, there is still a great need for effective antibodies to target CX3CR1. Our preliminary results show the potential of the CX3CR1 antibody produced by our group, against chronic and acute pancreatitis related pain. Further research is needed to evaluate its potential on targeting pancreatic cancer.

Keywords: CX3CR1, mAb, pancreatitis

[OP-17]

Characterization of tumor-infiltrating CD66b^{+} monocyte subset discloses NF-kB-mediated differentiation

Hamdullah Yanık[1], Kerim Bora Yılmaz[2], Timuçin Erol[3], Güneş Esendağlı[1]
[1]Department of Basic Oncology, Hacettepe University Cancer Institute, Ankara
[2]Department of General Surgery, University of Health Sciences, Gülhane Faculty of Medicine, Ankara
[3]Department of General Surgery, Hacettepe University Faculty of Medicine, Ankara, Turkey

Introduction: CD66b is a marker used to distinguish granulocytes from other myeloid cells. Our previous studies have shown that CD66b can also be detected on circulating monocytes of cancer patients and that these cells form a subpopulation with high inflammatory capacity. The aim of this study was to determine the factors that cause circulating monocytes to express CD66b and to correlate the presence of these cells in the tumor microenvironment with inflammatory factors.
Materials-Methods: CD14^{+}HLA-DR^{+}monocytes isolated from healthy donors were stimulated with IL-1β proinflammatory recombinant cytokine, which are frequently found in the tumor microenvironment, at different concentrations and durations. CD66b, CD80, CD86, LOX-1 surface markers were analyzed by flow cytometry. Then, monocytes were cultured with tumors and supernatants collected from tumors. Functional properties like T cell induction capacity and migration properties were also analyzed. Finally, the NF-κB pathway was silenced with BMS-345541 chemical blocking agent and IL-1β receptor antagonist and validation experiments were performed.
Results: In vitro and co-culture stimulation of CD14^{+}HLA-DR^{+} monocytes isolated from healthy subjects with tumor tissues from cancer patients resulted in a significant increase in the amounts of surface markers such as CD66b, LOX-1, CCRL2, CD80, CD86, PD-L1 which were determined to be specifically increased in the this monocyte subpopulation, compared to the non-stimulated control group. These cells were highly migratory and possessed higher capacity to activate T cells. IL-1β stimulation was found to be the factor causing the increase of this population in sterile inflammation. Blockade of the NF-κB pathway and IL-1β receptor interaction prevented CD66b expression resulting from these stimulations.
Conclusion: Factors inducing stimulation of the NF-kB pathway originating from the tumor microenvironment, particularly IL-1β, cause the development of a proinflammatory subpopulation in monocytes that can be characterized by CD66b expression.
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Keywords: Cancer, monocyte, macrophages, IL1B, NFKB

Poster Presentation

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[PP-002]

Essential Regulatory Role of PAK4 in T-Cell Activation

Feyza Kostak[1], Ismail Reisli[2], Suray Pehlivanoglu[1]
[1]Necmettin Erbakan University, Faculty of Science, Department of Molecular Biology and Genetics, Konya, Turkey.
[2]Necmettin Erbakan University, Faculty of Medicine, Department of Pediatric Immunology and Allergy, Konya, Turkey.

PAK4(p-21 Activated Kinase 4) is a serine/threonine kinase involved in cytoskeletal dynamics cell migration and proliferation. T-cell activation relies on costimulatory signals and cytokines, which are essential for immune regulation. Actin cytoskeleton remodeling and integrin-mediated migration are crucial for an effective immune response. This study investigates the potential regulatory role of PAK4 in T-cell activation. PBMCs were isolated from healthy donors and individualised to experimental set as follows: untreated cells, cells treated with PF-3758309(a PAK4 inhibitor), PHA(Phytohemagglutinin), and PF+PHA. Flow cytometry analysis using CD3/CD25, CD4/CD25, and CD8/CD25 activation markers was performed to examine T-cell activation. Additionally, cell viability was assessed using the MTT assay, and flow cytometry was employed to analyze cell cycle phases. Western blotting was conducted to measure the protein levels of PAK4, p-PAK4 (S474-phosphorylated PAK4) and α-tubulin. The results indicated that PHA stimulation resulted in enhanced PAK4 activation (3.2-fold), while PAK4 inhibition by PF-3758309 led to its suppression in PBMCs. However, the expression levels of endogenous PAK4 remained unaltered. Concurrently, α-tubulin expression exhibited a parallel response to PAK4 activation. MTT analysis showed that PHA induced the proliferation ability of the cells (4.05-fold), however, PAK4 inhibition reversed this effect to the baseline state. Cell cycle analysis demonstrated that the S-phase cell population increased with PHA(11.2-fold), while PAK4 inhibition, either alone (0.3-fold) or in combination with PHA(1.2-fold), decreased this. According to flow cytometric analysis, PHA strickingly led to increase in CD3+CD25+(40.4-fold), CD4+CD25+(23.4-fold) and CD8+CD25+(213.4-fold) cells. Despite the presence of PHA stimulation, PAK4 inhibition resulted in a significant decrease in various subsets of T lymphocytes, including a 3.7-fold decline in CD3+CD25+ cells, a 2.9-fold reduction in CD4+CD25+ cells, and a notable 5.3-fold decrease in CD8+CD25+ cells. In conclusion, the present findings suggest that PAK4 may act as a key regulatory factor in T cell activation and immune response modulation.

Keywords: PAK4, Phytohemagglutinin, T-Cell Activation

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[PP-003]

Retinoic Acid-Induced Gut-Homing Tissue Resident Memory T Cells: A Step Toward Precision Immunotherapy

Dicle Çalışkan[1], Irem Evcili[2], Buse Duru Aras[1], Bilgi Güngör[2]
[1]Izmir Biomedicine and Genome Center, Izmir, TÜRKİYE; Dokuz Eylul University, İzmir International Biomedicine and Genome Institute, Molecular Biology and Genetics Department, Izmir, TÜRKİYE
[2]Izmir Biomedicine and Genome Center, Izmir, TÜRKİYE

Tissue-resident memory T cells (TRM) protect against recurring infections and maintain long-term immunity in barrier sites. During antigen presentation in lymph nodes, dendritic cells (DCs) release mediators that shape TRM induction and distribution by imprinting tissue-specific homing moieties. One such mediator is all-trans retinoic acid (RA), a metabolite of vitamin A, which regulates the expression of gut-homing molecules integrin α4β7 and CCR9 in activated T cells. These molecules are essential for guiding T cells to the intestinal mucosa. In this study, we aimed to develop an *in vitro* model that mimics the natural dendritic cell-driven differentiation of TRMs. Using all-trans retinoic acid (RA) and TRM-inducing mediators such as TGF-β, we induced the differentiation of naive T cells into TRM-like cells with gut-homing characteristics (CCR9 and α4β7). We isolated naive T cells from C57BL/6 mice spleens using magnetic separation and stimulated them longitudinally with various doses of RA and/or TRM-inducing conditions. The stimulated cells were analyzed for TRM markers CD69 and CD103, as well as gut-homing molecules α4β7 and CCR9 via flow cytometry. We also measured TNF-α and IFN-γ concentrations from the culture supernatant using ELISA. We quantified the CCR9-dependent migration of stimulated cells using transwell assay. Our findings demonstrated that RA induced the differentiation of naive T cells into a gut-specific TRM phenotype by upregulating CCR9 and CD103 expressions. Furthermore, RA stimulation doubled CCR9-dependent migration, indicating enhanced gut-homing potential of in vitro differentiated TRMs. These results highlight the capacity of RA-mediated *in vitro* T cell conditioning in inducing tissue-specific memory T cells. This approach has the potential to improve targeted tissue-specific immunotherapies by enhancing the formation of TRM cells with a specific homing ability, which could be highly beneficial for T-cell-based therapies targeting cancer and infectious diseases.
This project is supported by TUBITAK 1001 Project (No:123R111)

Keywords: tissue resident memory T cells, all-trans retinoic acid, adoptive T cell transfer

[PP-004]

Investigating Dendritic Cell Subpopulations and *PTPN22* Gene Expression as Prognostic Biomarkers in Type 1 Diabetes Mellitus

Tamay Seda Taşçı[1], Nilgün Işıksaçan[2], Pınar Atar[3], Asuman Gedikbaşı[4], Suzin Tatonyan[5], Sevgi Kalkanlı Taş[1]
[1]Sağlık Bilimleri Üniversitesi, Hamidiye Sağlık Bilimleri Enstitüsü, İmmünoloji Anabilim Dalı, İstanbul, Türkiye
[2]Sağlık Bilimleri Üniversitesi, Bakırköy Dr. Sadi Konuk Eğitim ve Araştırma Hastanesi, Biyokimya Bölümü, İstanbul, Türkiye
[3]İstanbul Üniversitesi, Aziz Sancar Deneysel Tıp Araştırma Enstitüsü, İmmünoloji Ana Bilim Dalı, İstanbul, Türkiye
[4]İstanbul Üniversitesi, Çocuk Sağlığı Enstitüsü, Pediatrik Temel Bilimler Ana Bilim Dalı, İstanbul, Türkiye
[5]İstanbul Üniversitesi, Sağlık Bilimleri Lisansüstü Eğitim Enstirüsü, İmmünoloji Ana Bilim Dalı, İstanbul, Türkiye

Objective: This study seeks to investigate dendritic cell subtypes and the expression of a specific gene to elucidate the pathogenesis of Type 1 Diabetes Mellitus (T1DM). The primary purpose is to provide insights that could guide the prognosis and treatment strategies for T1DM patients.
Materials-Methods: The study groups included newly diagnosed patients or those diagnosed within the last five years, long-term diagnosed patients (>=5 years), and a control group comprising healthy individuals without known diseases. A total of 39 samples were analyzed for biochemical parameters (HbA1c and C-peptide), immunophenotyping, and gene expression studies. Immunophenotyping was performed using flow cytometry to assess dendritic cell subpopulations, and their distribution percentages were calculated. Additionally, qualitative reverse transcriptase PCR was employed to evaluate gene expression levels. The expression of the *PTPN22* gene (rs2476601), which is thought to be associated with immune system function, was analyzed. Relative quantitative molecular analyses were conducted using the mean Ct values of each group.
Results: The HbA1c levels in both newly diagnosed and long-term diagnosed groups were significantly higher than those in the control group. C-peptide levels were significantly lower in the newly diagnosed and long-term diagnosed groups compared to the control group. The mDC values in both patient groups were significantly lower than in the control group ($p<0.05$). In contrast, the mDC2 value in the long-term diagnosed group was considerably higher than in both the control and newly diagnosed groups. No statistically significant differences were observed among the groups in terms of relative gene expression levels.
Conclusion: The distribution of specific dendritic cell subpopulations and *PTPN22* gene expression levels have been identified as potential prognostic biomarkers in the progression of T1DM. It is possible that these findings will contribute to the early diagnosis and development of personalized treatment strategies.

Keywords: Autoimmune Diseases, Dendritic Cells, *PTPN22* Gene, Type 1 Diabetes Mellitus

[PP-005]

Biscotti-Twice-Baked-Cake is a New Product of the Milk Ladder in Children With Cow's Milk Allergy

Cemre Naz Bulbuloglu[1], Duygu Yazici[2], Gizem Kuruoglu[3], Nilgin Bahar[4], Mahir Serbes[4], Dilek Ozcan[4], Betul Buyuktiryaki[3], Cansın Sackesen[3]
[1]: Koç University Graduate School Of Healt Sciences, Koç University's Research Center For Translational Medicine.
[2]Swiss Institute of Allergy and Asthma Research (SIAF), Switzerland
[3]Koç University School of Medicine Pediatric Allergy and Immunology Department
[4]Cukurova University, Pediatric Allergy and Immunology Department

Objective: This study aims to evaluate a stepwise oral immunotherapy (OIT) approach for children with IgE-mediated cow's milk protein allergy (CMPA). By introducing milk in a controlled manner, starting with biscotti-twice-baked cake and progressing through once-baked cake, fermented milk, and whole milk, we aim to assess its role in increasing tolerance and reducing allergic reactions.
Method: Children with IgE-mediated CMA who presented to two pediatric outpatient clinics were included in the study. The reintroduction of milk into the diet was initiated with a biscotti. Participants who could tolerate 30g of biscotti (0.23g milk protein) were considered "biscotti tolerant" and advanced to the next stage, which involved consuming once-baked cake. Subsequently, they progressed to the fermented milk and whole milk stages. Each step was followed for at least one month.
Results: A total of 30 children with CMA [mean age=79.5 months (55.75–118 months)] participated in the study. Participants who reacted to Biscotti had higher levels of total IgE [458 (308-771) IU/L], milk-sIgE [71.4 (18-100)], and casein-sIgE [62.9 (16-79.5)] compared to non-reactive ones [total IgE=202 (84.8-493.5), milk-sIgE=22 (11.5-43.9), casein-sIgE=12.8 (8.2-28)] (p=0.11, p=0.086, p=0.069, respectively). Seventeen participants successfully completed the biscotti consumption phase, while 7 experienced a reaction. Six participants are still in the process of consuming biscotti. Among those who progressed to the once-baked cake stage, 10 were tolerant, and 1 showed a reaction. At the fermented milk product stage, 6 were tolerant, and 1 reacted. Finally, 6 participants successfully tolerated whole milk and were able to incorporate it into their diet.
Conclusion: The stepwise OIT approach, beginning with biscotti and progressing through baked and fermented milk to whole milk, appears to be a promising method for increasing milk tolerance in children with CMPA. Gradual exposure may enhance desensitization, reduce allergic reactions, and improve long-term outcomes.

Keywords: Milk Allergy, Oral Immunotherapy, Baked cake

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[PP-007]

Phenotypic and Functional Analysis of NK Cell Subsets in Behçet's Uveitis: Effects of Adalimumab Treatment and Comparative Inflammatory Responses with Axial Ankylosing Spondylitis

Fehim Esen[1], Aysenur Kokoglu[1], Cigdem Cetin[2], Ozlem Turkyilmaz[3], Nihan Aksu Ceylan[5], Merih Oray[5], Haner Direskeneli[6], Ilknur Tugal Tutkun[5], Ahmet Gul[4], Gunnur Deniz[1], Esin Aktas Cetin[1]
[1]Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Immunology, Istanbul, Turkey
[2]Istanbul University, Institute of Graduate Studies in Health Sciences, Istanbul, Turkey
[3]Istanbul Medeniyet University, Faculty of Medicine, Department of Ophthalmology, Istanbul, Turkey
[4]Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Rheumatology, Istanbul, Turkey.
[5]Istanbul University, Istanbul Faculty of Medicine, Department of Ophthalmology, Istanbul, Turkey
[6]Marmara University, School of Medicine, Department of Internal Medicine, Division of Rheumatology, Istanbul, Turkey

Introduction: The aim of this study was to investigate changes in NK cell subsets among Behçet's uveitis patients following adalimumab treatment.
Methods: The study included 14 Behçet's uveitis patients, 13 axial ankylosing spondylitis patients as diseased-controls, and 23 healthy controls. Blood samples were collected during active disease and upon clinical remission (at least 3 months post-treatment). Peripheral blood NK cell subsets were analyzed using flow cytometry, and plasma cytokine levels were measured using multiplex bead array.(Istanbul-Univ-TSA-35128, TUBITAK-120Z958)
Results: Significant improvements were observed in Behçet's uveitis patients' anterior chamber cells, angiography scores, visual acuity, and macular thickness, and in ankylosing spondylitis patients' BASDAI scores. Activating receptor NKG2D expression increased, while inhibitory receptor NKG2A decreased during active disease in both Behçet's uveitis and ankylosing spondylitis groups. After adalimumab treatment, NKG2D expression decreased and NKG2A increased in both groups. During active disease, Behçet's uveitis patients exhibited elevated intracellular expressions of IL-10, IL-4, TGF-β, and IL-17 compared to healthy controls, while ankylosing spondylitis patients showed higher expressions of IFN-γ, TNF-α, IL-4, TGF-β, and IL-17. Active Behçet's uveitis patients also had higher soluble plasma IL-2, IL-10, TNF-α, IFN-γ, and FasL levels compared to active ankylosing spondylitis. Post-adalimumab treatment, plasma levels of IL-2, TNF-α, IFN-γ, and FasL remained higher in Behçet's uveitis compared to ankylosing spondylitis patients, with a slight elevation in IL-10 levels.
Discussion: Both Behçet's disease and ankylosing spondylitis are MHC class-I associated inflammatory diseases with overlapping pathways. Adalimumab enhanced inhibitory but diminished activatory NK receptor responses. Reflecting GWAS data, active Behçet's uveitis patients exhibited heightened IL-10 and IL-17 responses (NKreg and NK17), while ankylosing spondylitis patients were more inclined to TNF-α and IFN-γ (NK1) responses. Adalimumab reinforced IL-10 in Behçet's uveitis, suggesting modulation of MHC/NK cell interactions. Systemic cytokine levels showed greater inflammation in Behçet's uveitis than ankylosing spondylitis, even after adalimumab treatment.

Keywords: Behçet, natural killer, adalimumab, uveitis

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[PP-008]

Telomere Length and Oxidative Stress in Multiple Sclerosis: Implications for Disease Progression

Hanbike Efe[1], Umut Voyvoda[1], Meziyet Dilara Reda[1], Sevim Naz Karisik[1], Erokay Gurz[2], Zeynep Tonbul[1], Batuhan Savsar[3], Elif Celik[2], Ugur Uygunoglu[4], Melih Tutuncu[4], Aksel Siva[4], Eda Tahir Turanli[5]
[1]Department of Molecular Biology and Genetics, Graduate School of Natural and Applied Science, Acibadem University, Istanbul, Türkiye
[2]Department of Translational Medicine, Institute of Health Science, Acibadem University, Istanbul, Türkiye
[3]Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Acibadem University, Istanbul, Türkiye
[4]Deparment of Neurology, Istanbul University Cerrahpaşa School of Medicine, Istanbul, Türkiye
[5]Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Acibadem University, Istanbul, Türkiye; Institute of Natural and Applied Science, Acibadem University, Istanbul, Türkiye

Objective
Multiple sclerosis (MS) is a demyelination disease characterized by neuroinflammation and neurodegeneration. Increasing evidence suggests a link between disease progression and biological aging. This study evaluates absolute telomere length (aTL) in CD19+ B cells, CD4+ CD25+ regulatory T (Treg) cells and peripheral blood mononuclear cells (PBMCs) and assesses oxidative stress levels in MS patients compared to healthy controls.

Methods
CD19+ B cells, CD4+ CD25+ Treg cells were enriched via magnetic affinity cell sorting. DNA was extracted from enriched CD19+ B cells, CD4+ CD25+ Treg cells, and total PBMCs of MS patients (aged 50±5) and age-matched healthy controls. Telomere length was measured using qPCR. MS patients were categorized into three groups according to Expanded Disability Status Scale (EDSS) score: mild (EDSS 0-2.5), moderate (EDSS 3-5), and severe (EDSS 5.5+). Oxidative stress levels were assessed using the TBARS method in serum samples. Statistical analyses were performed to compare telomere length and oxidative stress levels across groups.

Results
Telomere length (aTL) was significantly shorter in all MS patients compared to healthy controls across all three cell types($p<0.0001$). Within MS patients, aTL showed a decreasing trend with increasing EDSS scores. In three subgroups of MS patient telomere length was significantly shorter in Tregs compared to both B cells($p<0.05$) and PBMCs($p<0.05$). Also, oxidative stress levels were significantly higher in MS patients compared to healthy controls($p<0.01$). Subtype analysis revealed that oxidative stress was markedly elevated in secondary progressive MS (SPMS) patients compared to relapsing-remitting MS (RRMS) patients ($p<0.0001$).

Conclusions
These findings suggest that telomere shortening in immune cells is linked to MS progression and may serve as a marker of biological aging. Increased oxidative stress in MS patients, particularly in SPMS, may contribute to accelerated telomere attrition. These results highlight the potential role of telomere dynamics and oxidative stress in MS pathophysiology and disease progression.

Keywords: Multiple Sclerosis, Telomere Length, Oxidative Stress, EDSS, Disease Progression, Biological Aging

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[PP-009]

Pharmacogenomic Analysis in Turkish Familial Multiple Sclerosis Cohort: Associations Between HLA Alleles and Disease-Modifying Therapies

Meziyet Dilara Reda[1], Alper Bülbül[2], Turkish Familial Ms Consortium[3], Aksel Siva[3], Melih Tütüncü[3], Uğur Uygunoğlu[3], Eda Tahir Turanlı[4]
[1]Department of Molecular Biology and Genetics, Graduate School of Natural and Applied Science, Acibadem University, Istanbul, Türkiye
[2]Department of Biostatistics and Bioinformatics, Graduate School of Health Sciences, Acibadem University, Istanbul, Türkiye
[3]Department of Neurology, İstanbul University Cerrahpaşa Faculty of Medicine, İstanbul, Türkiye
[4]Department of Molecular Biology and Genetics, Faculty Of Engineering and Natural Sciences, Acibadem University, Istanbul, Türkiye; Institute of Natural and Applied Science, Acibadem University, Istanbul, Türkiye

OBJECTIVE
Multiple sclerosis (MS) is an immune-mediated disease of the central nervous system, shaped by genetic and environmental factors. Treatment aims to manage symptoms and utilize disease-modifying therapies (DMTs) to reduce MS activity, with no treatment completely eliminating the disease. This study explores genetics' role in DMT efficacy, highlighting critical markers like human leukocyte antigen (HLA) alleles for personalized treatment. Despite links between genetic variants and drug responses, results are often complicated. Future research should focus on clarifying MS's genetic landscape through advanced genomic techniques and larger cohorts, aiming to analyze pharmacogenomic studies of treatment variability and clinical outcomes.
METHOD
This study examined genetic variants in the Turkish Familial Multiple Sclerosis (TuFaMS) cohort to understand their link to DMT responses. Whole exome sequencing was performed to identify variants. We filtered known MS treatment variants and novel ones, then used Fisher's exact test via SciPy to evaluate the associations between drug use and allele presence.

RESULTS
Variants linked to DMTs for MS include HLA risk variants: DQA1*01:02, DQB1*06:02, DRB1*15:01, DQA1*03:01, and DQB1*03:02. An analysis of 138 WES data from TuFaMS assessed medication switches and genetic correlations. Fisher's exact test indicated significant associations between drug-HLA allele pairs. The strongest link was between Fingolimod and DQB1*06:02 ($p = 0.0166$), with 5 individuals showing both. Ocrelizumab had a near-significant association with B*07:02 ($p = 0.0626$), while Fingolimod was linked to DRB1*15:01 ($p = 0.0675$). Natalizumab showed trends with DQA1*03:01 ($p = 0.0680$) and DQB1*03:02 ($p = 0.1292$), suggesting genetic influences on drug response.

CONCLUSION
Our findings indicate that certain HLA variants, including DQB1*06:02, DRB1*15:01, B*07:02, DQA1*03:01, and DQB1*03:02, may affect responses to therapies like Fingolimod, Ocrelizumab, and Natalizumab in MS, suggesting the potential for personalized treatments through pharmacogenomic profiling.

Keywords: Multiple Sclerosis, Disease Modifying Therapies, Pharmacogenomic, HLA Variant

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[PP-010]

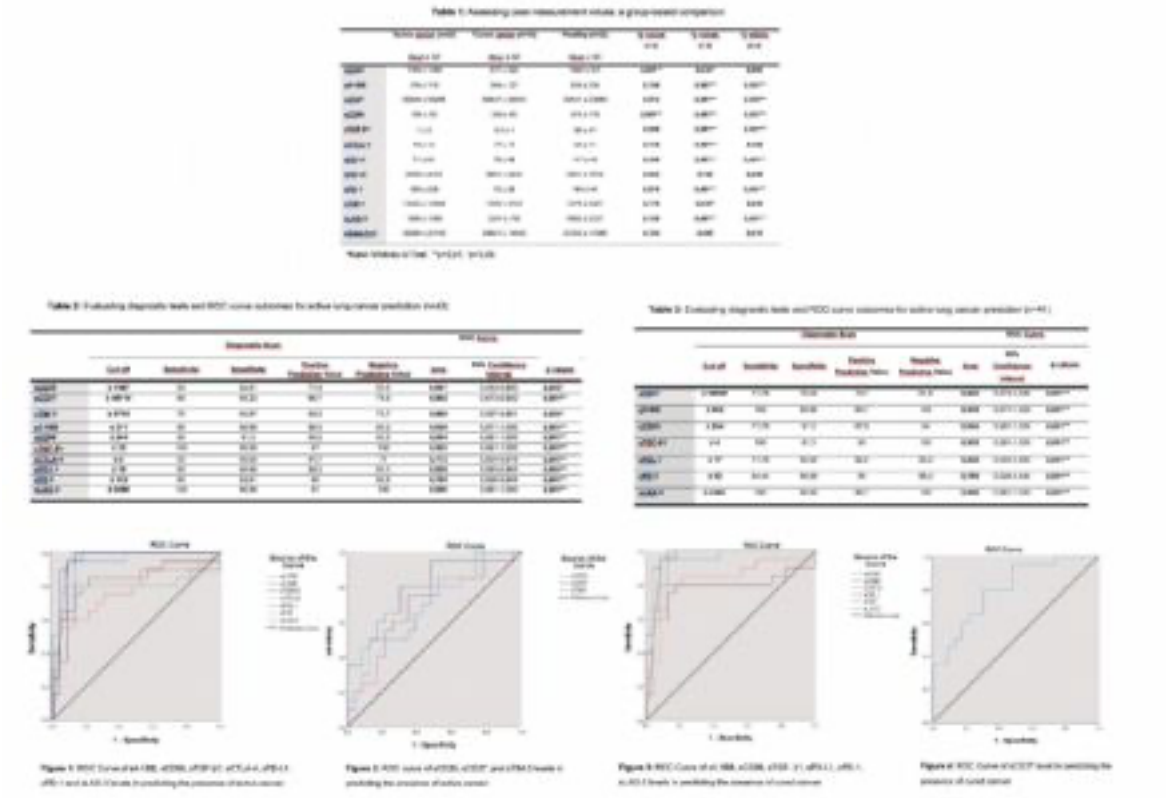
Soluble Immune Checkpoint-Related Proteins as Biomarkers in Lung Cancer: Diagnostic and Prognostic Insights

Nurgul Naurzvai[1], Aysenur Kokoglu[1], Gulsah Hikmet Tanyildiz[1], Suleyman Temiz[2], Leyla Ozer[2], Zeynep Gural[3], Serap Yucel[3], Yildiz Okuturlar[5], Nilgun Yilmaz Demirci[4], Gul Gursel[4], Gunnur Deniz[1], Esin Cetin Aktas[1]
[1]Department of Immunology, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Istanbul, Turkiye
[2]Department of Medical Oncology, Acibadem Hospital Group, Turkiye
[3]Department of Radiation Oncology, Acibadem University Hospital, Istanbul, Turkiye
[4]Department of Respiratory Diseases, Gazi University Hospital, Ankara, Turkiye
[5]Department of Internal Medicine, Acibadem University Hospital, Istanbul, Turkiye

Introduction: Lung cancer remains a leading cause of cancer-related mortality, highlighting the need for effective diagnostic and therapeutic strategies. Immune checkpoint (IC) inhibitors have revolutionized cancer treatment; however, their efficacy is often limited by the complex and heterogeneous nature of tumors. This study investigates the role of soluble IC-related proteins (sICPs) as potential biomarkers for lung cancer diagnosis, prognosis, and treatment response.
Method: Plasma samples from 20 active lung cancer patients, 18 recovered lung cancer patients, and 23 healthy controls were analysed. Plasma levels of sCD25, s4-1BB, sCD27, sCD86, sTGF-β1, sCTLA-4, sPD-L1, sPD-L2, sPD-1, sTIM-3, sLAG-3, and sGalectin-9 were measured using the LEGENDplex assay with flow cytometry. Statistical significance of differences between groups was assessed using the Mann-Whitney U test, with p-values <0.05 considered significant. Additionally, ROC curve analysis and correlation analysis were conducted to evaluate the diagnostic performance and relationships between clinical factors and biomarker levels.
Results: Active cancer patients had higher sCD25 levels compared to recovered and healthy individuals. Both active and recovered cancer patients had lower levels of s4-1BB, sCD86, sTGF-β1, sCTLA-4, sPD-L1, sPD-1, and sLAG-3 compared to healthy individuals. sCD27 levels were higher in both active and recovered cancer patients than in healthy individuals. Additionally, active cancer patients had higher sTIM-3 levels compared to healthy individuals. Logistic regression models incorporating these biomarkers demonstrated high accuracy in distinguishing lung cancer patients from healthy controls (Figure 1 and Table 1).
Discussion: Our study identifies several sICP with significant differences between active cancer patients, recovered cancer patients, and healthy individuals, providing potential diagnostic tools for cancer detection and monitoring. Additionally, significant correlations between specific clinical factors and biomarker levels offer further insights into the disease's progression and treatment response.

Keywords: Immune checkpoint inhibitors, Soluble immune checkpoint-related proteins, Lung cancer diagnostic and prognostic markers, Small cell lung cancer, Non small cell lung cancer, Cured lung cancer

Tables and Figures



Correlations between disease stages and treatments with soluble markers

Group	Correlation Pair	N	r	p
Active Disease Group				
	Lymph node and sPD-L2	17	0.629	0.007
	Stage and sB7-2/CD86	17	0.668	0.003
	Secondary tumour and sB7-2/CD86	19	-0.501	0.029
	Recent immunotherapy and sCD27	13	0.592	0.033
	Recent immunotherapy and sTIM-3	13	0.592	0.033
	Previous immunotherapy and sPD-1	13	0.66	0.014
	Diabetes mellitus and s4-1BB	11	-0.693	0.018
Cured Group				
	Operation and sCD86	18	0.47	0.021
	Secondary tumour and sLAG-3	17	-0.423	0.044

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[PP-011]

Identification of PD-L1+ B cells in Non-Small Cell Lung Cancer (NSCLC) and investigation of molecular mechanisms of their effect on antitumoral response

Oğuzhan Köse[1,2], Akif Turna[3], Melek Ağkoç[4], Turan Demircan[5], Deniz Duralı[1,2]
[1]Cancer Research Center, Research Institute for Health Sciences and Technologies (SABITA), Istanbul Medipol University, Istanbul,Türkiye
[2]Department of Medical Microbiology, Istanbul Medipol University, Istanbul, Türkiye
[3]Department of Thoracic Surgery, Istanbul University - Cerrahpasa, Istanbul, Türkiye
[4]Department of Thoracic Surgery, Erzurum City Hospital, Erzurum, Türkiye
[5]Muğla Sitki Kocman University, Department of Medical Biology, Mugla, Türkiye

NSCLC remains the leading cause of cancer-related deaths worldwide. The tumor microenvironment in NSCLC is a complex environment composed of a variety of immune and non-immune cells that collectively shape disease progression and therapeutic response. Given PD-L1's role as a key TME regulator, we focused on PD-L1+ B cells to assess their immunosuppressive potential and clinical impact in NSCLC.

A total of 55 patients with the diagnosis of stage IB to IV NSCLC were enrolled. Samples from tumors, mediastinal lymph nodes, healthy lung tissue and peripheral blood were collected from all patients. Lymphocytes were isolated from the blood and the tumor mass. B lymphocyte PD-L1 expressions and characterizations are determined by flow cytometry. RT-qPCR was used to measure EBI3, IL-12p35 IL-10, LGALS1 and MAF mRNA expression.

The number of tumor-infiltrating (TIL) B cells (13.89%±1.45%) was significantly higher than those in healthy lung tissue (4.36%±0.63%) ($p<0.0001$). Similarly, PD-L1 and PD-1 expressions of TIL B cells (9.63%±1.81% and 8.62%±1.78%, respectively) were elevated compared to that of healthy lung tissue (3.39%±0.58% and 1.97%±0.86%, respectively) ($p=0.0018$ and $p=0.0028$, respectively). Flow cytometric analyses revealed that PD-L1+ B cells are found in the "terminally differentiated" group, which includes memory and plasma cells, compared to PD-L1- B cells and healthy lung tissue, however there is no statistical significance ($p=0.0815$ and $p=0.4171$, respectively).
All analyzed genes were up-regulated in PD-L1+ TIL B cells compared to PD-L1- cells: EBI3 (3.96-fold), IL-12p35 (10.87-fold) IL-10 (9.79-fold), LGALS1 (1.14-fold) and MAF (96.73-fold). However, only IL-10 and LGALS1 expression levels were statistically significant when compared to healthy donors ($p=0.0228$ and $p=0.0162$, respectively).

The gene expression profile of PD-L1+ B cells suggests an immunosuppressive function in anti-tumor immunity. These findings indicate that tumor-infiltrating PD-L1+ CD19+ B cells exhibit regulatory B cell characteristics. However, modus operandi of this effect remains to be unveiled.

Keywords: NSCLC, Breg, Immunoregulation, PD-L1

[PP-012]

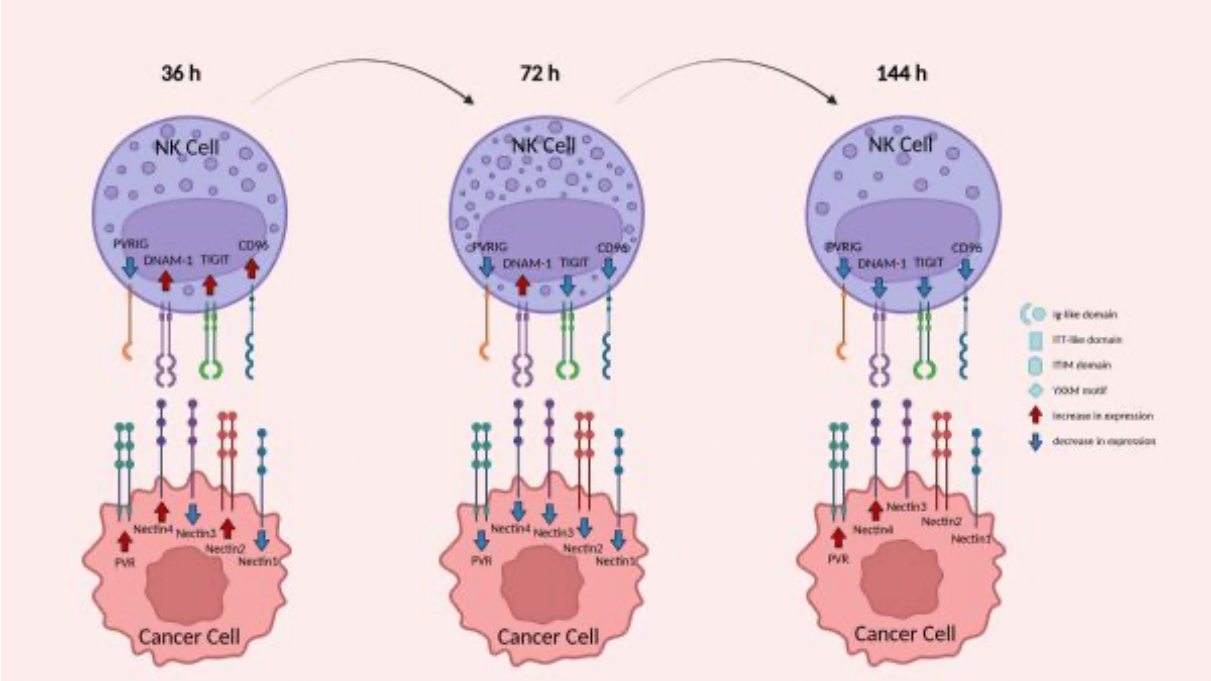
The Relationship Between Nectin Family Molecules and NK Cell Functions in Pancreatic Cancer

Rukiye Abanoz, Melek Günindi Korkut, Begüm Kocatürk, Füsun Özmen
Department of Basic Oncology, Hacettepe University Cancer Institute, Ankara, Türkiye

Objective
Pancreatic cancer has one of the highest mortality rates due to late diagnosis and limited treatment options. The tumor microenvironment shapes tumor progression and immune response through the complex interaction of immune cells and stromal components. NK cells constitute an important defense mechanism against tumor cells through their cytotoxic activity. However, the immunosuppressive microenvironment of pancreatic cancer may limit the functionality of NK cells and contribute to tumor immune escape. In this study, we aim to mechanistically investigate the interactions of NK cells with pancreatic cancer and evaluate these interactions in terms of therapeutic targets.
Materials and Methods
The interaction of NK-92 cells and pancreatic cancer cell line BxPC-3 was studied in a six-day co-culture model. During the co-culture process, gene expression levels of Nectin family and PVR molecules were analyzed by qRT-PCR, NK cell receptors (DNAM-1, TIGIT, PVRIG and CD96) and cytotoxicity molecules (CD107a, Perforin, Granzyme B) were analyzed by flow cytometry.
Results
DNAM-1 family receptors and ligands were observed to exhibit variable expression levels at different time points during co-culture. Expression of the activation receptor DNAM-1 increased during the first five days, whereas expression of the inhibitory receptors TIGIT and CD96 tended to decrease from 36 hours onwards. PVRIG expression decreased over time. The cytotoxicity of NK cells remained high until 72 hours, but decreased after this time. Accordingly, it was observed that the expression of PVR increased in parallel with DNAM-1 activation receptor, while it was inversely correlated with the inhibitory receptors TIGIT and CD96.
Conclusions
Our study reveals that DNAM-1 family receptors and ligands interact in a complex manner and that NK cell functions are regulated by these relationships. In conclusion, the PVR molecule, a common ligand of DNAM-1, TIGIT and CD96 receptors, plays an important role in the regulation of NK cell functions.

Keywords: NK cell, Pancreatic Cancer, Nectin Family, PVR, DNAM-1 Family

Interactions between NK and cancer cells



Receptor and ligand interaction of NK cells and BxPC-3 cells. At 36 h of co-culture, NK cells became activated, increasing their cytotoxicity and triggering ligand expression in target cells. By 72 h, DNAM-1 expression continued to increase while the expression levels of other receptors and ligands decreased. From 72 h to 144 h, PVR and Nectin-4 expression increased, while other ligands remained unchanged and NK cell receptor expression decreased.

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[PP-013]

Myeloid cell dynamics in primary breast tumors and lung metastases following chemotherapy: an immunological emphasis on drug resistance

Sibel Goksen[1], Zeynep Ergul Ulger[2], M. Cisel Aydın Mericoz[3], Gunes Esendagli[4]
[1]Department of Medical and Surgical Research,Hacettepe University Institute of Health Sciences, Ankara, Turkiye
[2]Department of Neurology, Hacettepe University School of Medicine, Ankara, Turkiye
[3]Department of Pathology, Koc University, Istanbul, Turkiye
[4]Department of Basic Oncology, Hacettepe University Cancer Institute, Ankara, Turkiye

Chemotherapy resistance remains a significant challenge in breast cancer treatment, driving tumor progression and metastasis. It also modulates the immune microenvironment, influencing treatment responses. This study investigates the dynamics of myeloid cell populations in primary and metastatic sites of doxorubicin-resistant and sensitive breast cancer models at early and late stages of tumorigenesis following treatment with doxorubicin or pemetrexed. In the early stage, $CD206^{+}FR\beta^{+}$ macrophages were significantly increased in the doxorubicin-resistant tumor model compared to the non-resistant model; however, their numbers declined in the late phase within primary tumor tissue. In metastatic lung tissue, macrophages were dominant cell population, whereas granulocytes progressively increased and became the dominant population in the late phase. Additionally, alveolar macrophage numbers were markedly reduced in the resistant model. Early chemotherapy administration reduced metastatic burden in both models compared to controls. A comparison between early and late chemotherapy treatments revealed a predominance of macrophages in the early phase. P-glycoprotein (P-gp), a key mediator of chemotherapy resistance, was highly expressed at the early stage; however, its expression was endured only under chemotherapy. These findings indicate that chemotherapy resistance induces significant alterations in the immune microenvironment, with myeloid cell dynamics playing a crucial role in shaping treatment responses. The observed reduction in metastatic burden with early chemotherapy and the marked changes in P-gp expression imply the need for novel immunomodulatory strategies to overcome chemotherapy resistance.

Project supported by Hacettepe University (No: TSA-2021-19541)

Keywords: Drug resistance, chemotherapy, $CD206^{+}FR\beta^{+}$ macrophages

[PP-017]
Immunophenotyping of HLA-DR$^+$T lymphocytes in ovarian cancer patients

Isilay Akdag Aras[1], Sila Uluturk[1], Ozhan Ozdemir[2], Vildan Altahhan[2], Vefa Sakar[2], Kerim Bora Yilmaz[3], Gunes Esendagli[1]
[1]Department of Basic Oncology, Hacettepe University, Ankara, Türkiye
[2]Department of Obstetrics and Gynecology, University of Health Sciences, Gulhane Research and Training Hospital,Ankara, Türkiye
[3]Department of General Surgery, University of Health Sciences, Gulhane Research and Training Hospital,Ankara, Türkiye

Objectives: HLA-DR, a member of the MHC-II protein family, primarily presents antigens to CD4$^+$ T lymphocytes.It also serves as a late activation marker on various T lymphocytes, particularly Cytotoxic T Cells (CTLs) in the tumor microenvironment.High-level HLA-DR expression in CTLs correlates with therapy response and may improves adoptive T cell therapy.This study focuses on ovarian cancer, in which information on HLA-DR$^+$ TILs in tumors and ascites is limited, a rapidly progressing disease treated with surgery and chemotherapy, with PD-1/PD-L1 inhibitors approved for both new and relapsed cases.Our aim is to explore the phenotype and functions of HLA-DR$^+$CD8$^+$ and CD4$^+$ TILs in the tumor microenvironment, ascites, and blood, and assess their potential as biomarkers for immunotherapy response.
Methods: PBMCs were isolated from blood and ascites of ovarian cancer patients, and blood from healthy donors.TILs and tumor cells were obtained from ovarian tumors using physical and chemical digestion.Expression of HLA-DR and ICIs on memory T cell subsets and Tregs was assessed via surface and nuclear staining,respectively,and analyzed by flow cytometry.
Results and Conclusions: HLA-DR expression was higher in ovarian cancer patient blood compared to healthy donors,with CD8$^+$ T cells expressing more HLA-DR than CD4$^+$ T cells.PD-1 expression was also elevated in patient blood on both T cell subsets, while TIM-3, LAG-3, and CTLA-4 showed no significant expression in either group.Importantly, HLA-DR$^+$ cells had significantly higher PD-1 expression than HLA-DR$^+$ cells on both CD4$^+$ and CD8$^+$ T cells.TEM and TEMRA memory subsets showed higher HLA-DR expression in patients than healthy donors.Although HLA-DR expression in ascites did not exceed blood levels,TILs had higher HLA-DR,PD-1,and other ICIs.Notably, HLA-DR$^+$ TILs showed elevated ICIs, specially PD-1 expression.In TIL memory subsets,TRM cells had the highest HLA-DR expression, followed by TEM and TCM cells.Tregs expressed similar levels of HLA-DR in blood and ascites but had the highest expression in the tumor microenvironment.

Keywords: ***T cells, HLA-DR, Ovarian cancer, ICIs, Memory***

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[PP-018]

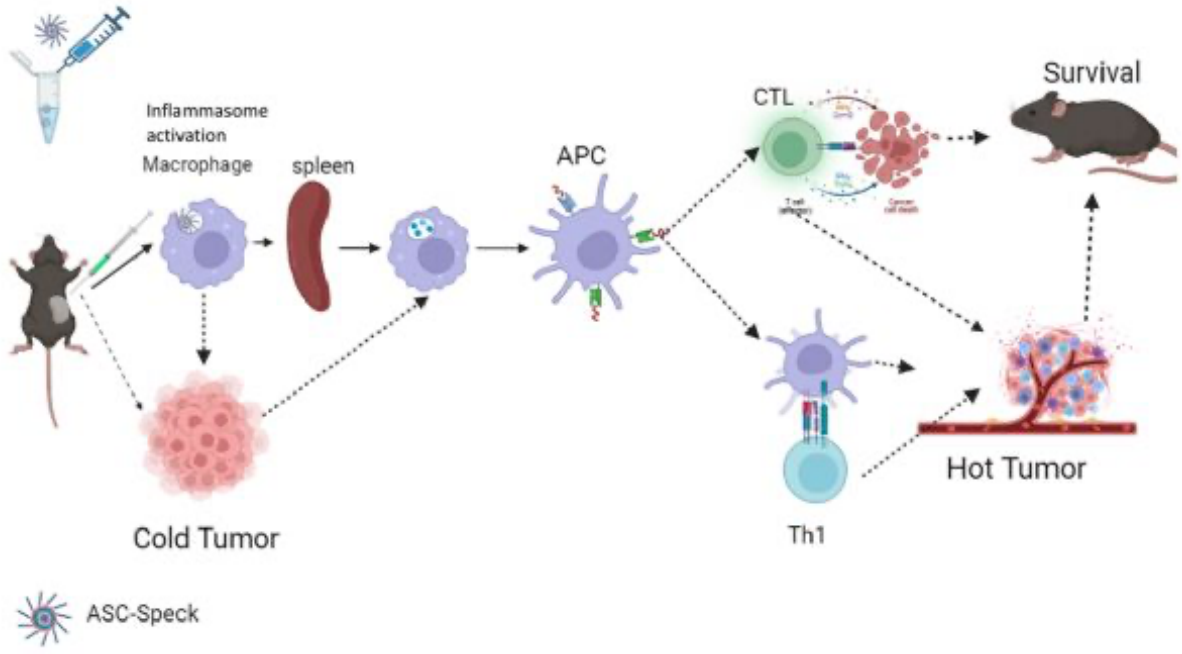
Targeting Of Tumor Associated Antigens Using A Novel Microparticle Based Vaccine Designe And Its Effects On The Tme

Davod Khalafkhany[1], Nesrin Erkiol[2]
[1]Akil lab, Molecular Biology Department, Bogazici University, Istanbul, Turkey
[2]Vaccizone LLC, Istanbul, Turkey

Introduction
ASC is an adaptor protein essential for inflammasome complex formation, which regulates cytokine secretion following the detection of PAMPs and DAMPs via NOD-like receptors. Overexpression of human ASC in HEK293 cells induces the formation of supramolecular complexes (ASC specks) that can sequester hydrophobic cytosolic peptides. Furthermore, antigen-ASC fusion proteins form similar stable microparticles intracellularly. These microparticles have inflammatory properties which we aim to use here as a novel carrier/adjuvant base for stable antigen delivery and vaccines [1, 2].
Results and Discussion
Systemic administration of mCherry-ASC microparticles in WT mice led to accumulation in the spleen and Peyer's patches for over two weeks. This accumulation was blocked by AMD3100, a CXCR4 antagonist that inhibits macrophage migration. Treatment with cOVA-ASC microparticles eradicated EG7-cOVA thymoma in 30% of animals and significantly increased anti-cOVA IgG titers compared to pure cOVA protein. Prophylactic administration of human MAGE-A3-ASC microparticles prevented B16 tumor development in 40% of mice. A trivalent vaccine combining hMAGE-A3, mMage-A1, and mMlana prevented melanoma in 66% of animals. Therapeutic administration of hMAGE-A3-ASC microparticles to mice with established B16 tumors resulted in tumor shrinkage or elimination in 40% of cases. TME analysis revealed increased infiltration of CD45+ immune cells and CD80+ M1 macrophages in tumors treated with antigen-ASC immunotherapy.
Conclusion
Tumor-associated antigen-carrying ASC microparticles enhance the immune response, demonstrating their potential for use both prophylactic and therapeutic cancer treatments in murine models by enhancing tumor infiltration of immune cells and changing the TME from immunologically cold to immunologically hot TME.

Keywords: Vaccine, TME, Biomarker

A suggestion of action mechanism of antigen-ASC microparticle on tumor.



[PP-020]

Characterization of exosomes derived from leuko-reduced red-blood cell concentrates and determination of their impact on T cell proliferation

Salih Haldun Bal[1], Diğdem Yöyen Ermiş[2], Gözde Arslan[3], Ceren Esen[3], Yağmur Aydın Atalay[3], Gizem Aytoğu[4], Kadir Yeşilbağ[4], Yasemin Herper[5], Haluk Barbaros Oral[2]
[1]Department of Immunology, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey, Dr. Rasit Durusoy Blood Bank, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey, Experimental Animal Breeding and Research Unit, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey, Experimental and Molecular Immunology Laboratory (Experim), Faculty of Medicine, Bursa Uludag University, Bursa, Turkey
[2]Department of Immunology, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey, Experimental and Molecular Immunology Laboratory (Experim), Faculty of Medicine, Bursa Uludag University, Bursa, TurkeyExperimental Animal Breeding and Research Unit, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey,
[3]Department of Immunology, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey, Department of Medicine-Immunology, Institute of Health Science, Bursa Uludag University, Bursa, TurkeyExperimental and Molecular Immunology Laboratory (Experim), Faculty of Medicine, Bursa Uludag University, Bursa, TurkeyExperimental Animal Breeding and Research Unit, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey,
[4]Department of Virology, Faculty of Veterinary Medicine, Bursa Uludag University, Bursa, Turkey
[5]Dr. Rasit Durusoy Blood Bank, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey, Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey

Objective: Leukoreduction removes leukocytes from red blood cell concentrates(RBCs) by filtration, thereby aiming to reduce transfusion-related immunomodulation(TRIM). However,RBC-derived exosomes accumulate during storage, potentially influencing immune responses post-transfusion. Herein, we investigated the effect of leukoreduction and different storage time on T cell proliferation to better understand TRIM mechanisms.
Materials-Methods: Voluntarily donated RBCs were either subjected to leukoreduction(LR) or left non-reduced (NR). Each group was divided into three separate blood bags and stored at +4°C for 0,21, and 42 days. Accumulating exosomes during storage were isolated by ultracentrifugation. Exosomal protein concentration, purity, size distribution, and tetraspanin expression were evaluated by micro-BCA assay, Western blotting, TRPS, and flow cytometry, respectively. Exosomes from each group(LR-D0,LR-D21,LR-D42 and NR equivalents) were pooled(LRp and NRp) and used at concentrations of 0.2µg, 1µg, and 5µg in CFSE-based T cell proliferation assays with PBMCs from healthy donors, either allogeneic or autologous.
Results: Micro-BCA results indicated that leukoreduction lowers exosome secretion(NR-D0, NR-D21, NR-D42, LR-D0, LR-D21, LR-D42 were 32.6; 299.1; 379.0; 29.8; 48.0; and 93.9 µg/mL, respectively). Western blot analysis confirmed the presence of exosomal markers Flotillin-1 and CD9, while the negative control Calnexin was absent. Size distribution ranged from 87 to 103 nm. Flow cytometry showed positivity for tetraspanins CD63(58.4%) and CD81(95.6%). Proliferation assays revealed that exosomes from LRp suppressed T cell proliferation more than NRp, particularly at 5 µg concentrations in both allogeneic and autologous conditions. This suppressive effect was primarily mediated by CD4+T cells.
Conclusion: These findings support previous evidence suggesting that leukoreduction alone may be insufficient to prevent TRIM. Exosomes, particularly those derived from leukoreduced RBCs, could contribute to immunomodulatory effects seen after transfusion, highlighting their potential role in TRIM development. This study was supported by TUBITAK under the Grant Number 222S919 and Bursa Uludag University Research Project Coordination Office under the Grant Number TAY-2022-6001.

Keywords: Exosome, Tranfusion, TRIM

[PP-021]

Outer Membrane Vesicle Conjugated RBD Vaccine Against COVID-19

Harun Önler, Ismail Cem Yılmaz, Muzaffer Yıldırım, Tuğçe Canavar Yıldırım, Ibrahim Tokmak, Zeynep Arzum, Elif Sena Özkoç, Mehmet Ali Öktem, Seyhan Içier, Ayça Özkan, Mehmet Inan, Ihsan Gürsel
İzmir Biomedicine and Genome Center

The COVID-19 pandemic, caused by the SARS-CoV-2 virus, has underscored the critical necessity for safe, effective, and scalable vaccines to control viral transmission and mitigate its profound global impacts. The receptor-binding domain (RBD) of the SARS-CoV-2 spike protein is instrumental in mediating viral entry into host cells and is thus a primary target for neutralizing antibodies, making it an attractive candidate for vaccine design. In this study, we aimed to develop a novel outer membrane vesicle (OMV)-based vaccine platform by conjugating the RBD antigen to the surface of lipopolysaccharide (LPS)-free OMVs. This strategy seeks to harness the inherent immunogenic properties of OMVs to elicit robust immune responses against SARS-CoV-2.
To achieve this, we expressed the RBD antigen utilizing the yeast-based Pichia pastoris expression system, ensuring proper folding and glycosylation. OMVs were produced and purified from an LPS-null Escherichia coli strain, ensuring safety and minimizing inflammatory responses associated with endotoxins. Conjugation of the omicron variant RBD antigen to OMVs was successfully accomplished using the highly specific SpyTag/SpyCatcher system, and the resulting vaccine candidate underwent extensive purification via multimodal chromatography. The immunogenic potential of the purified vaccine candidate was rigorously assessed using enzyme-linked immunosorbent assay (ELISA).
Our immunological evaluations demonstrated that this OMV-RBD vaccine formulation elicited potent humoral responses, as evidenced by significantly elevated antigen-specific antibody titers. Additionally, robust cell-mediated immunity was observed, as measured by enzyme-linked immune absorbent spot (ELISPOT) assays. Importantly, strong virus-neutralizing antibody (VNA) responses were detected, substantially outperforming both placebo and control groups.
In conclusion, our results indicate that the OMV-based vaccine incorporating the omicron RBD via the SpyTag/SpyCatcher conjugation platform effectively stimulates both humoral and cellular immune responses. This innovative vaccine candidate holds substantial promise as a potent preventive strategy against COVID-19, warranting further investigation and development.

Acknowledgement: This work is supported by TUBITAK 1004 Project (No:22AG013)

Keywords: COVID-19 pandemic, SARS-CoV-2, Vaccine development, Outer membrane vesicles (OMVs), OMV Vaccines

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[PP-022]

Leishmania major Extracellular Vesicles Mediate Long-Term Protection Against Cutaneous Leishmaniasis

Ibrahim Tokmak[1], Ismail Cem Yılmaz[2], Muzaffer Yıldırım[2], Elif Sena Özkoç[2], Irem Evcili[2], Bilgi Güngör[2], Volkan Yazar[3], Bekir Salih[4], Ülkü Güler[4], Ihsan Gürsel[2], Mayda Gürsel[2]
[1]Izmir Biomedicine and Genome Center, Izmir, TURKIYE; Dokuz Eylul University, Izmir International Biomedicine and Genome Institute, Department of Molecular Biology and Genetics, Izmir, TURKIYE
[2]Izmir Biomedicine and Genome Center, Izmir, TURKIYE
[3]Bilkent University, Faculty of Science, Department of Molecular Biology and Genetics, Ankara, TURKIYE
[4]Hacettepe University, Faculty of Science, Department of Chemistry, Ankara, TURKIYE

Introduction
Leishmaniasis is a vector-borne disease caused by various Leishmania protozoan parasites. Depending on the infecting species and host factors, the disease manifests as cutaneous, mucocutaneous, or visceral leishmaniasis. As a neglected tropical disease, leishmaniasis poses a significant global health problem, necessitating the urgent need for effective vaccines to control its spread.

Objectives & Methods
Here, we investigated the immunoprotective efficacy of *Leishmania major*-derived extracellular vesicles (Lm-EVs) in a murine model of cutaneous leishmaniasis. Lm-EVs were isolated and purified from axenically cultured metacyclic promastigotes under GMP-adaptable, scalable, cost-effective conditions and subsequently characterized. 6–8-week-old BALB/c mice were immunized with Lm-EVs, and the humoral immune response was evaluated by measuring soluble Leishmania antigen (SLA)-specific antibody levels using ELISA at four months post-immunization. To assess the immunoprotective potential of the Lm-EVs, mice were challenged with live luciferase-expressing transgenic *L. major* parasites four months post-vaccination. Parasite loads were monitored using in vivo imaging system (IVIS), longitudinally.

Results & Conclusion
Lm-EV-immunized groups demonstrated significantly higher SLA-specific antibody titers compared to the placebo group, with sustained levels observed even after four months post-immunization. Notably, Lm-EV-immunized mice showed ~ 4,5 fold reduction in parasite loads significantly, post-challenge compared to the placebo group. These results indicate that Lm-EVs harbor immunogenic antigens capable of eliciting durable protective immune responses, highlighting their potential as a standalone candidate vaccine against cutaneous leishmaniasis.

Acknowledgement
This work is partially supported by TUBITAK, 1004-22AG013 and 1003-115S073 grants awarded to IG and MG, respectively.

Keywords: *Leishmania major, extracellular vesicles, vaccine, cutaneous leishmaniasis*

[PP-023]

Dysregulation of hsa-miR-124-3p and Galectin-13 as Potential Immunoregulatory Biomarkers in HIV Infection

Ilker Inanc Balkan[1], Andleeb Bukhari[2], Haktan Sönmez[2], Burhaneddin Oktan[2], Muhammet Fatih Akdemir[2], Bilgül Mete[3], Fehmi Tabak[3], Günnur Deniz[1], Umut Can Küçüksezer[1]
[1]Istanbul University, Aziz sancar Institute of Experimental Medicine, Department of Immunology
[2]Istanbul University-Cerrahpasa, Cerrahpasa Faculty of Medicine, Department of Medical Pharmacology
[3]Istanbul University-Cerrahpasa, Cerrahpasa Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology

Objective: In the present study, we aimed to investigate the difference in hsa-miR124-3p before and after antiretroviral treatment (1, 2 and 6 month) and its correlation with immunomodulatory biomarker (Galectin-13/PP13) and immunological (CD4 count) marker.
Materials-Methods: Blood samples were drawn at different time points from healthy volunteers and naïve HIV patients. The blood was centrifuged for plasma extraction and stored at -80°C for further analysis. RT-qPCR and ELISA techniques were used for miRNA and various proinflammatory biomarker analysis. CD4 count was analyzed using flow cytometry. Statistical analysis was done using Python and Graphpad Prism.
Results: In our study, we demonstrated a significant increase in the expression of hsa-miR-124-3p in HIV positive subjects than that of healthy individuals. We found a significant downregulation in the hsa-miR-124-3p expression of month 1, 2 and 6 post treatment when compared with HIV treatment naïve group ($p < 0.001$). Treatment-naive HIV-positive patients showed a significant upregulation of hsa-miR-124-3p compared to the control group ($p < 0.001$). Galectin-13/PP13 levels were significantly lower in HIV treatment-naïve individuals compared to healthy individuals ($p < 0.0001$). However, PP13 levels gradually increase with antiretroviral treatment duration, reaching near-normal by the sixth month ($p < 0.0001$, $p < 0.01$). We found that galectin-13/PP13 and CD4 count exhibited a positive correlation with treatment duration.
Conclusion: This study identified hsa-miR-124-3p dysregulation before and after antiretroviral therapy, emphasizing its potential role in HIV pathogenesis. Additionally, this is the first study to investigate galectin-13 expression in HIV-positive individuals, revealing significant downregulation. Given its involvement in apoptosis modulation via JNK and p38-MAPK activation and PI-3K-Akt/ERK1/2 suppression, galectin-13 may serve as a valuable biomarker for assessing immune status and ART response. These findings provide insights into the immunoregulatory mechanisms in HIV and warrant further investigation to understand their clinical relevance.

Keywords: HIV, miRNA, CD4+ T count, hsa-miR124-3p, galektin-13, PP13

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[PP-024]

Cross-Neutralization of Hazara Virus by Convalescent Sera from CCHFV-Infected Individuals

Özlem Bakangil[1], Mehmet Ziya Doymaz[2]
[1]Beykoz Institute of Life Sciences and Biotechnology & Department of Biotechnology, Institute of Health Sciences, Bezmialem Vakıf University, Istanbul, Türkiye
[2]Beykoz Institute of Life Sciences and Biotechnology & Department of Medical Microbiology, Faculty of Medicine, Bezmialem Vakıf University, Istanbul, Türkiye

Crimean Congo Hemorrhagic Fever Virus (CCHFV), a member of the family Nairoviridae and genus Orthonairovirus, is a zoonotic pathogen with a negative-sense, single-stranded RNA genome. It causes severe hemorrhagic fever in humans, with case fatality rates of up to 40%. Research on live CCHFV is restricted to Biosafety Level-3 (BSL-3) laboratories due to its high pathogenicity, creating barriers to broad scientific investigation and slowing the development of antivirals, immunotherapies, and vaccines. Hazara Virus (HAZV), closely related to CCHFV within the same family and genus, shares considerable genetic, structural, and antigenic features, particularly in its nucleoprotein and polymerase. Unlike CCHFV, HAZV is non-pathogenic in humans and can be safely studied in BSL-2 laboratories, making it an attractive surrogate model for CCHFV research. In this study, we investigated whether convalescent sera from individuals recovered from CCHFV infection could neutralize HAZV. HAZV was propagated in multiple cell lines to generate high-titer viral stocks. Microneutralization assays were then performed with these sera. Following incubation, viral replication was assessed using real-time PCR (RT-PCR). The results revealed effective neutralization of HAZV by CCHFV convalescent sera, indicating strong antigenic cross-reactivity between the two viruses. These findings support the utility of HAZV as a safer, more accessible surrogate model for studying CCHFV. Using HAZV in BSL-2 settings can broaden participation in research, facilitating the study of immune responses, antiviral testing, and vaccine development. This approach has the potential to accelerate the discovery of effective countermeasures against CCHFV, ultimately strengthening global health preparedness and response efforts.

Keywords: Crimean-Congo Hemorrhagic Fever Virus (CCHFV), Hazara Virus (HAZV), Cross-Neutralization

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[PP-025]

Modulation of Human Macrophage Polarization and Phagocytic Function by a New Polyherbal formulation *NOQ19*

Asli Korkmaz[1], Duygu Sag[2]
[1]Izmir Biomedicine and Genome Center; Department of Genome Sciences and Molecular Biotechnology, Izmir International Biomedicine and Genome Institute, Dokuz Eylul University, Izmir, Turkey
[2]Izmir Biomedicine and Genome Center; Department of Medical Biology, Faculty of Medicine, Dokuz Eylul University; Department of Genomic Sciences and Molecular Biotechnology, Izmir International Biomedicine and Genome Institute, Dokuz Eylul University, Izmir, Turkey

Objective: *NOQ19* is a new polyherbal formulation containing 19 ingredients derived from 13 medicinal plants. While there is limited research on *NOQ19*, pre-clinical and clinical investigations have shown that the herbs in *NOQ19* separately have many beneficial effects, including antiviral and antibacterial. India has licensed *NOQ19* for managing mild to moderate COVID-19 due to its promising outcomes in clinical trials. However, the impact of *NOQ19* on the immune system is not known. The aim of this study is to investigate the impact of *NOQ19* on the polarization and function of human macrophages.
Methods: Human blood monocyte-derived macrophages were pre-treated with 500 µg/mL *NOQ19* extract for 2 hours, then polarized into M1 (LPS+IFNγ), M2a (IL-4), or M2c (IL-10) macrophages for 22 hours. M1/M2 polarization markers were analyzed by RNA-seq, qPCR, flow cytometry, and ELISA. Phagocytosis was analyzed using pHRodo-conjugated heat-killed *Staphylococcus aureus* bioparticle.
Results: *NOQ19* increased the expression of the M1 markers IL-1β, CCL5, CXCL5, NOTCH3, and C3, while decreasing the expression of the M2 markers CD163, IL-10, MS4A6A, SELENOP, and F13A1 at mRNA level. The surface expression of the M1 marker CD64 and the M2 markers CD200R, CD209, and CD163 was decreased in M0, M2a, and M2c macrophages. *NOQ19* decreased the levels of secreted IL-10 and TGF-β in all macrophage groups. Lastly, *NOQ19* treatment decreased the phagocytic capacity of both polarized and unpolarized macrophages.
Conclusion: *NOQ19* modulates human macrophage polarization and phagocytic function with a downregulation of receptors which have distinct roles in phagocytosis. Recent studies indicate that CD209 may serve as a potential new entry receptor for SARS-CoV-2 by facilitating endocytosis. Thus, *NOQ19* could be a promising new supplement to modulate macrophage polarization and function for the treatment of viral infections, such as SARS-CoV-2.

This study is funded by TUBITAK ARDEB 1001 grant (no: 222S331) to D.S.

Keywords: Medicinal plants, macrophage polarization, inflammation, viral infections, SARS-CoV-2

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[PP-026]

The Value of Ferroptosis Components As Biomarkers In NBIA

Selen Soylu[1], Nuray Sancar[1], Elif Dilsiz[1], Melike Tezel[1], Vuslat Yılmaz[1], Erdem Tüzün[1], Ibrahim Kamacı[2], Şeyma Çiftçi Aytaç[3], Ayşegül Gündüz[4], Sezin Baslo[5], Cem Ismail Küçükali[1], Zuhal Yapıcı[2]
[1]Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Neuroscience, Istanbul, Türkiye
[2]Istanbul University, Istanbul Faculty of Medicine, Department of Neurology, Istanbul, Türkiye
[3]Ege University, Faculty of Medicine, Department of Neurology, İzmir, Türkiye
[4]Istanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine, Department of Neurology, Istanbul, Türkiye
[5]Prof. Dr. Mazhar Osman Mental Health and Neurological Diseases Training and Research Hospital, Neurology Clinic, Istanbul, Türkiye

Objective
Neurodegeneration with Brain Iron Accumulation (NBIA) comprises a group of heterogeneous neurodegenerative disorders characterized by iron accumulation in specific brain regions, particularly the globus pallidus and substantia nigra. The role of ferroptosis, a form of regulated cell death associated with oxidative stress due to iron accumulation, in the pathogenesis of NBIA remains unclear. This study aimed to evaluate ferroptosis biomarkers in NBIA patients to elucidate their contribution to the disease pathophysiology.
Materials and Methods
Serum and cerebrospinal fluid (CSF) samples were collected from patients diagnosed with three major NBIA syndromes: MPAN (n=16; age=28±11), BPAN (n=7; age=14±17), and PKAN (n=7; age=20±9), as well as from healthy controls (n=13; age=13.1±3.62). The levels of ferroptosis biomarkers, including DPP4, GPx4, sTfR1, and hepcidin—the key regulator of iron metabolism—were measured using the ELISA method.
Results
A significant decrease in serum GPx4 levels was observed in the BPAN group ($p=0.0130$), while there were no significant differences in DPP4 ($p=0.0503$) and sTfR1 ($p=0.1094$) levels between groups. Additionally, hepcidin levels in the serum of NBIA patients were markedly lower ($p<0.0001$). In the MPAN group, CSF samples revealed a significant increase in sTfR1 levels ($p=0.0259$).
Conclusion
Contrary to expectations, the findings indicate a decrease in GPx4, a key ferroptosis marker, and lower hepcidin levels, which play a crucial role in iron homeostasis. These results suggest that ferroptosis may have a potential role in the pathogenesis of NBIA.

Keywords: ferroptosis, iron accumulation, oxidative stress

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[PP-027]

Successful management of hypereosinophilia syndrome with targeted therapy: A case report

Semih Ayten[1], Nurgul Naurzvai[2], Ant Uzay[3], Yildiz Okuturlar[1], Sami Suleyman Karti[3]
[1]Department of Internal Medicine, Acıbadem University School of Medicine, Istanbul, Turkey
[2]Department of Immunology, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Istanbul, Turkey; Department of Respiratory Medicine, Acibadem University Hospital, Istanbul, Turkey
[3]Department of Medical Hematology, Acibadem University Hospital, Istanbul, Turkey

Introduction: Hypereosinophilia syndrome (HES) is a rare hematologic disorder characterized by persistent eosinophilia and organ damage, with a prevalence of less than 1 in 100,000 individuals. HES is classified into primary (neoplastic), secondary (reactive), and idiopathic forms. The identification of specific genetic mutations, such as PDGFRA/FIP1L1, has improved the understanding and management of HES, allowing for targeted therapies.

Case Presentation: A 46-year-old male presented with weakness, oral and genital ulcers, weight loss and night sweats. Further examination splenomegaly, cervical and supraclavicular lymphadenopathy and pleural effusion were detected. A peripheral blood eosinophil count of 10,460 cells per microliter was observed. A bone marrow biopsy and genetic tests, including FISH for BCR-ABL and PDGFRA/FIP1L1 fusion transcripts, were investigated. Due to severe eosinophilia, the patient was started on prednisolone, hydroxyurea, and allopurinol. A diagnosis of HES was established, and based on the results indicating BCR-ABL negativity and PDGFRA/FIP1L1 positivity, treatment with imatinib, an inhibitor of multiple tyrosine kinases, was initiated. During the follow up, laboratory values were assessed following treatment with Imatinib, revealing a significant improvement in the eosinophil count and the overall hematologic profile, alongside a notable reduction in symptoms such as weakness, ulcers, and night sweats; furthermore, imaging studies conducted post-treatment indicated a marked decrease in lymphadenopathy and pleural effusion, underscoring the therapeutic efficacy of Imatinib in managing the patient's condition.

Discussion: This case highlights the importance of a thorough diagnostic workup in patients presenting with complex symptoms and significant eosinophilia. The identification of PDGFRA/FIP1L1 fusion transcripts was crucial in diagnosing HES and guiding targeted therapy with imatinib. The patient's positive response to imatinib underscores the efficacy of targeted therapy in managing HES. This case underscores the need for awareness and prompt diagnosis of HES to initiate appropriate treatment and improve patient outcomes.

Keywords: Hypereosinophilia Syndrome (HES), Imatinib, Eosinophilia, BCR-ABL

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[PP-028]

Low-density neutrophils display characteristics of myeloid-derived suppressor cells (MDSC) in cancer but not in acute inflammation: a comparative analysis in humans and mice

Ece Tavukçuoğlu[1], Kerim Bora Yılmaz[2], Utku Horzum[1], Diğdem Yöyen Ermiş[1], Ayşegül Üner[3], Güneş Esendağlı[1]
[1]Hacettepe University Cancer Institute, Department of Basic Oncology, Ankara
[2]University of Health Sciences, Diskapi Yildirim Beyazit Research and Training Hospital, Department of General Surgery, Ankara
[3]Hacettepe University, Faculty of Medicine, Department of Pathology, Ankara

Objective: In this study, it was aimed to investigate phenotypical and functional characteristics of splenic low-density neutrophils in cancer and acute inflammation.
Materials-Methods: An isolated spleen injury model in mice was established where the abdominal organs were revealed by surgery, several incisions were introduced onto the spleen and the surgical wound on the abdominal wall was closed through sterile stitching. As a control group, the mice underwent sham operation with abdominal incision and wound closure only. Additionally, 4T1 tumor model was used as a tumor-bearing mice model. Mice splenocytes and peripheral blood samples were layered over 1.077 g/mL. Ly6G+ granulocytes were isolated by using MACS and FACS. Spleen samples were collected from the gastric or pancreatic cancer patients or trauma patients who underwent splenectomy. Human splenocytes and peripheral blood samples were layered over 1.077 g/mL and 1.119 g/mL Ficoll. Low-density neutrophils were enriched by CD66b MACS and FACS.
Results: The granulocytes derived the tumor-bearing mice significantly suppressed the T cell proliferation whereas those isolated from the trauma model or the sham-operated animals did not. Although the level of granulocytes increased to comparable levels in both acute spleen trauma model and in cancer model; however, only the granulocytes from the tumor-bearing mice displayed myeloid-derived suppressor cells (MDSC) functions. Low-density neutrophils from cancer patients' spleen possessed high ROS production capacity, STAT3 expression and T cell suppression which are clearly associated with the characteristics of MDSC. Low-density neutrophils from trauma patients were identified with high Nrf2 expression, enhanced phagocytosis activity and did not suppress T cell responses.

Conclusions: Overall, this is the first report showing that chronic inflammation is required for low-density neutrophils to acquire suppressive properties and display MDSC functions in spleen.
This study was supported by TÜBİTAK (project no: 220S701).

Keywords: Neutrophil, inflammation, cancer, myeloid-derived suppressor cells

[PP-029]

Rapid detection of Cryptococcus

Rabiye Altınbaş, Nazlı Gürkan, Muhammed Nurullah Açıkgöz
Gaziantep City Hospital, Gaziantep, Turkiye

Cryptococcosis is a significant cause of meningitis, particularly in HIV-AIDS patients, transplant recipients, and individuals with defects in cell-mediated immunity. Early diagnosis and appropriate antifungal therapy are life-saving in cryptococcal meningitis cases, as is the case with other fungal infections. The polysaccharide capsule, along with the presence of laccase and phospholipase enzymes, are major virulence factors of Cryptococcus spp. However, epidemiological data regarding cryptococcosis in our country remain limited (1). Therefore, determining the burden of cryptococcosis in tertiary hospitals will provide valuable contributions to the existing literature. Advances in PCR diagnostic technologies have facilitated the early and accurate diagnosis of meningitis and encephalitis cases. Multiplex PCR technology allows for the rapid detection of multiple pathogens in a single reaction (2). At our institution, we utilize the QIAstat-Dx Meningitis/Encephalitis Panel, a multiplex PCR method, to identify pathogens responsible for meningitis and/or encephalitis. In this retrospective study, 193 cerebrospinal fluid samples were analyzed for Cryptococcus gattii/neoformans. PCR test results were negative (0.0%) for all patients. Although higher prevalence rates have been reported in some studies, the absence of clinically reported cryptococcal meningitis cases in our country is consistent with our hospital data, supporting the conclusion that the incidence of cryptococcosis remains low in our region. In conclusion, reducing Cryptococcus-related mortality relies on early diagnosis and the effective management of underlying conditions. Similar studies conducted and published periodically by other centers will be beneficial for both our region and our country.

Keywords: Cryptococcus gattii/neoformans, immunosuppressed, molecular diagnosis

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[PP-030]

Process Optimization for Large-Scale Recombinant Human Interleukin-2 Production in an *E. coli* Expression System

Beyza Nur Uysak[1], Elif Çelik[2], Haydar Demir[3], Ozan Fidan[3], Erdem Bozluolcay[4], Tolga Sütlü[5]
[1]Acıbadem Mehmet Ali Aydınlar University, Graduate School of Natural and Applied Sciences, Department of Molecular Biology and Genetics, Istanbul, Türkiye
[2]Acıbadem Mehmet Ali Aydınlar University, Graduate School of Health Sciences, Department of Translational Medicine; Epsilon Electronics Industry and Trade, Istanbul, Türkiye
[3]Epsilon Electronics Industry and Trade, Istanbul, Türkiye
[4]MNT Health Services and Trade Inc, Istanbul, Türkiye
[5]Acıbadem Mehmet Ali Aydınlar University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics, Istanbul, Türkiye

Objective: Interleukin-2(IL-2) is a crucial cytokine that plays a key role in immune regulation. Because of its vital functions, IL-2 is widely used in the treatment of cancer and autoimmune diseases. Furthermore, IL-2 is the most crucial cytokine used in the preparation of cell therapy products such as CAR-T and CAR-NK cells. Due to the growing demand for human IL-2 in research and clinical applications, recombinant human IL-2(rhIL-2) is produced to ensure a reliable and scalable supply. Given its significant therapeutic potential, cost-effective and efficient production methods are essential. Our study focuses on optimizing large-scale rhIL-2 production using *Escherichia coli*, providing a practical and economical approach.

Methods: Codon-optimized IL-2 gene with C-terminal his-tag was cloned into a bacterial expression vector and introduced into *E. coli*BL21(DE3) cells. Expression of the protein was induced using isopropyl β-D-1-thiogalactopyranoside(IPTG). The bacterial culture was cultivated up to 7 liters under optimized conditions in an automated bioreactor system to promote maximum protein expression in large-scale culture. After cultivation, cells were harvested and lysed with sonication and freeze-thaw cycling to obtain purified inclusion bodies. Inclusion bodies were solubilized using denaturants, and his-tagged rhIL-2 was purified and refolded simultaneously by FPLC using a HisTrap column.

Results: Our results demonstrated that the efficient and scalable expression of rhIL-2 was feasible using BL21(DE3) cells. OD600 value of cultures reached over 30 with fed-batch culturing and bacterial pellet mass reached over 40g/L. Inclusion bodies were isolated from the bacterial pellets and solubilized efficiently. Purification and refolding via chromatography were confirmed through SDS-PAGE analysis, revealing a distinct rhIL-2 protein band with over 95% purity.

Conclusion: Large-scale rhIL-2 production in *E. coli*and its purification with on-column refolding were optimized for efficient protein production. Our results demonstrate the feasibility of efficient and scalable rhIL-2 production.

Acknowledgement: This project is supported by Epsilon Electronics Industry and Trade, and TÜBİTAK-TEYDEB 1501(No:3230734).

Keywords: Interleukin-2 (IL-2), *E. Coli*, protein expression, bioreactor system

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[PP-031]

Development of synthetic non-immunoglobulin recombinant adhiron binders using yeast surface display

Görkem Odabaş[1], Ilgın Akkaya[1], Alp Ertunga Eyüpoğlu[2], Batu Erman[1]
[1]Department of Molecular Biology and Genetics, Acibadem University, Istanbul, Turkey
[2]Department of Molecular Biology and Genetics, Bogazici University, Istanbul, Turkey

The majority of therapeutic molecules are antibodies whose production relies on immunization and hybridoma technology. Naïve antibody display libraries constructed from non-immunized organisms can replace hybridoma technology as they "by-pass" immunization. Their complex structure and poor tissue penetration are problems of antibodies. Non-immunoglobulin recombinant binders are advantageous over antibodies as they are not limited by the immune system and can be easily expressed in bacteria. Thermostable Adhirons are derived from plant cysteine protease inhibitors and have two loop regions that can be engineered. To this end, we have constructed a synthetic yeast surface display library for the development of recombinant Adhirons against different target proteins.
Adhiron variants were generated by randomizing the loop regions. Diversified Adhirons and a yeast expression vector were co-transformed to S. cerevisiae to generate a library of 1×10^7 yeast transformants by homologous recombination. This library was screened against model proteins (Streptavidin, PATZ1-BTB and CRP) using magnetic bead and FACS selections. First round of magnetic selection started with induced yeast cells and protein coated beads. Binders were magnetically isolated, cultured overnight and induced for the next rounds of selection. Consecutive magnetic selections started with depletion steps against empty beads followed by a positive selection step using protein coated beads. Magnetically enriched cells were FACS sorted with increased stringency by decreasing protein concentrations. To identify clones yeast expression plasmids were sequenced. Streptavidin screening yielded two unique clones. Identified Adhiron proteins were expressed in E. coli and were purified by NiNTA affinity and size-exclusion chromatography. Affinities of Adh-SA.1 and Adh-SA.2 were assessed using SPR and found to be 1.1 µM and 230 nM respectively.
Here, we constructed a new synthetic YSD Adhiron library without any prior immunization step and showed that isolated binders can be produced from bacterial cells in high yields at low cost in contrast to antibodies.

Keywords: Yeast Surface Display (YSD), Adhiron Library, Synthetic Binders, Streptavidin

[PP-033]

Long Non-Coding RNAs as Regulators of CD4+ T Cell Activation: Insights from Bulk and Single-Cell Transcriptomic Analyses

Cagatay Sahin, Emre Kilic, Kadir Ziya Durmus, Hüseyin Atakan Ekiz
İzmir Institute of Technology

Immunotherapies such as checkpoint inhibitors and adoptive T cell transfer rely on effective T cell activation. Identifying CD4+ T cells and factors influencing their activation is crucial. Long non-coding RNAs (lncRNAs, >200 bp) have emerged as potential regulators of T cell responses, but their role under different stimulatory conditions remains underexplored.

This study analyzed CD4+ T cell microarray data (GSE39594) from human blood under three conditions—non-stimulated, anti-CD3, and anti-CD3/CD28—at 0, 4, and 24 hours (three replicates each). Differential gene expression analysis using Limma in R identified lncRNAs associated with activation. Target lncRNAs were further examined using single-cell RNA sequencing (Seurat) to assess their tissue-specific expression.

Bioinformatics analysis revealed that CD3/CD28 stimulation led to stronger CD4+ T cell activation than CD3 alone. Differential expression analysis identified numerous lncRNAs with distinct activation patterns, suggesting a role in modulating T cell responses. Single-cell data further highlighted tissue-specific lncRNA expression and its potential impact on T cell function. Several lncRNAs emerged as potential biomarkers, with some enriched in specific T cell subsets.

These findings underscore the importance of lncRNAs in CD4+ T cell activation and immune regulation. Understanding their tissue-specific roles could improve immunotherapy strategies and targeted T cell modulation in disease contexts.

Keywords: Immunotherapy, oncoimmunology, long non-coding RNA, cancer

[PP-034]

LncRNA Landscape of T Cells During Activation and Antitumor Immunity

Emre Kılıç, Çağatay Şahin, H. Atakan Ekiz
İzmir Institute of Technology, Molecular Biology and Genetics, İzmir, Turkiye

T cells are essential for eliminating malignant cells and infected cells in immune responses. Studies on T cell activation have advanced our understanding of biological processes like phenotypic shifts, intracellular signaling, and T cell diversity. While protein-coding genes have been heavily studied, the role of long non-coding RNAs (lncRNAs) in modulating T cell responses remains under-explored. LncRNAs, defined as RNA transcripts longer than 200 nucleotides with no open reading frames, influence gene regulation, cellular differentiation, and various physiological functions. Given the distinct characteristics of T cell subsets and multifaceted biological roles of lncRNAs, it is likely that both protein-coding and non-coding RNAs contribute to shaping the T cell phenotypes. In this study, we aimed to investigate the lncRNA expression landscape during T cell activation on bulk RNA sequencing data. We analyzed publicly available transcriptomic data (GSE197067), where pan-T cells from healthy donors were activated with anti-CD3/CD28 beads at multiple time points. Differential gene expression analysis was performed using the limma package in R, followed by gene set enrichment analyses and pathway visualizations. Furthermore, T cell activation-specific gene expression profiles were validated using single-cell RNA sequencing (scRNA-seq) data from CD4+ and CD8+ within the tumor microenvironment. Our analysis identified over 2,500 lncRNAs, with approximately 600 exhibiting significant differential expression across activation time points. Some lncRNAs displayed transient expression changes, whereas others consistently up- and downregulated upon activation. These lncRNAs later analyzed in tumor microenvironment scRNA-seq dataset to examine their relevance in tumor-infiltrating T cells, especially CD4+ and CD8+ subpopulations. Specific lncRNAs showed variable expression patterns between in vitro activation and within the tumor microenvironment. In summary, this study highlights the heterogeneity in lncRNA expression in various T cell populations. By intersecting the bulk and scRNAseq data, we provide novel insights into lncRNA expression dynamics in T cell activation and antitumor immunity.

Keywords: Pan-T cell activation, Long non-coding RNAs, Tumor Microenvironment, Differential expression analysis

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[PP-035]

Investigation of CD4+, CD8+, and T Follicular Helper Cells in Follicular Lymphoma Patients

Fatih Akboga[1], Melis Irem Kuyas[1], Fehmi Hindilerden[2], Ipek Yonal Hindilerden[3], Emine Gulturk[2], Meliha Nalcaci[3], Gunnur Deniz[1], Metin Yusuf Gelmez[1]
[1]Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Immunology, Istanbul, Türkiye
[2]Istanbul Dr. Sadi Konuk Training and Research Hospital, Department of Internal Medicine, Department of Hematology, Istanbul, Türkiye
[3]Istanbul University, Istanbul Medical Faculty, Division of Medical Sciences, Department of Internal Medicine, Istanbul, Türkiye

INTRODUCTION
Follicular lymphoma (FL) is an indolent B-cell non-Hodgkin lymphoma and originated from germinal center B cells of the lymphoid follicle. The immune status of patients is critical in the pathogenesis and progression of FL. This study aimed to investigate the roles of $CD4^+$,$CD8^+$, and T follicular helper (TFH) cells in the pathogenesis of FL.
METHOD
Peripheral blood mononuclear cells (PBMCs) were isolated from the whole blood of FL and healthy controls. The cells were stimulated with PMA/ionomycin for 5 hours. The levels of PD-1, TIM-3, LAG-3, TIGIT,IL-4, IL-17,IL-21,IFN-γ, perforin, and granzyme-B in $CD4^+$,$CD8^+$, and TFH cells were analyzed by flow cytometry.
RESULTS
In FL patients, a decrease in $CD4^+$T cells and increase in $CD8^+$T and TFH cells were observed. Compared to healthy subjects, IL-4 and IFN-γ levels were elevated in $CD4^+$ and $CD8^+$T cells but reduced in TFH cells. Conversely, IL-21 levels were found to be decreased in both $CD4^+$ and TFH cells. While there was no significant difference in granzyme B levels, perforin levels in $CD8^+$T cells were increased. TIM-3 and TIGIT expression was higher in $CD4^+$ and $CD8^+$T cells of patients compared to healthy controls. Following treatment, a reduction in PD-1, TIM-3, and TIGIT levels in $CD4^+$T cells, a decrease in PD-1 levels in $CD8^+$T cells, and a decline in IL-4, TIM-3, and TIGIT levels in TFH cells were observed.
DISCUSSION
The increase in TFH cells may promote the survival and proliferation of lymphoma cells. Although there is an increase in the number of $CD8^+$T cells and their perforin levels, the elevated expression of TIM-3 and TIGIT in these cells suggests an exhausted phenotype, potentially leading to insufficient anti-tumor responses. Treatment may help strengthen immune responses by reducing the expression of immune checkpoint molecules in T cells.

This project was supported by Istanbul University-Scientific-Research-Projects-Unit-(project no: TSA-2022-39113).

Keywords: Follicular Lymphoma, CD4+ T Cells, CD8+ T Cells, T Follicular Helper Cells, TFH

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[PP-037]

CD8+ T Cell-related Inhibitory Molecules in Behçet's Disease: Comparison with Myasthenia Gravis

Seyda Bilgin[1], Taylan Kaplan[5], Rabia Deniz[6], Arman Çakar[3], Hacer Durmuş[3], Yeşim Parman[3], Fatma Alibaz Öner[5], Ahmet Gül[4], Haner Direskeneli[5], Güher Saruhan Direskeneli[2]
[1]Department of Immunology, Institute of Health Sciences; Department of Physiology Istanbul Medical Faculty, Istanbul University
[2]Department of Physiology, Istanbul Medical Faculty, Istanbul University
[3]Department of Neurology, Istanbul Medical Faculty, Istanbul University
[4]Department of Rheumatology, Istanbul Medical Faculty, Istanbul University
[5]Department of Rheumatology, Faculty of Medicine, Marmara University
[6]Department of Rheumatology, University of Health Sciences, Istanbul, Turkey

Introduction:
Regulatory molecules such as killer-cell immunoglobulin-like receptor (KIR) or programmed cell death protein-1 (PD-1) on CD8+T cells may effect disease development through inhibition of other cells.On the other hand, type I interferons are tried in autoimmune and autoinflammatory diseases to induce regulatory mechanisms.
Objectives:
To examine CD8+ T cells for regulatory molecules in Behçet's disease (BD) and to compare with a classical autoimmune disease such as myasthenia gravis (MG).
Methods:
Peripheral mononuclear cells (PBMCs) of BD (n=37), MG patients (n=29) and age- and sex-matched healthy controls (HCs, n=24) were compared for PD-1, total KIR, as well as CD28, TIGIT, CD45RA and CCR7 molecules on CD3+CD8+ T cells.Furthermore, the cells from patients and HCs were stimulated in vitro by anti-CD3 and type I interferon (IFN-α/2α1b) for 4 days and the induction of PD-1, TIM-3 and TIGIT on CD8+T cells was compared.
Results:
PD-1+CD8+T cells with potentially regulatory functions were significantly lower in BD patients, regardless of disease activity, than in HCs, whereas in MG group, as the diseased-control, no difference was observed.CD3 stimulation of PBMCs induced PD-1 expression in all groups, whereas addition of type I IFN on stimulated CD8+T cells produced differential responses: The increase of PD-1 on CD8+ T cells in healthy as well as diseased controls was not observed in BD patients. The responsive increase of similar co-inhibitory molecules, TIM-3 and TIGIT, in HCs was not seen in BD patients.
Although KIR+CD8+T cells were significantly increased in MG, BD patients did not reveal any difference compared to HCs for these cells.
Conclusion:
The decrease of PD-1 on CD8+T cells, along with decreased PD-1, TIM-3 and TIGIT responses of CD8+T cells upon type I interferon stimulation in BD suggest dysregulated functions of these molecules in BD which are not observed in the diseased control with autoimmune origin.

Keywords: Immune dysfunction, immune regulation, T cells, BD, MG

[PP-038]

Regulatory Features of CD8+ T Cells in Myasthenia Gravis

Seyda Bilgin[1], Arman Çakar[3], Fikret Aysal[4], Hacer Durmuş[3], Yeşim Parman[3], Güher Saruhan Direskeneli[2]
[1]Department of Immunology, Institute of Health Sciences; Department of Physiology, Istanbul Medical Faculty, Istanbul University
[2]Department of Physiology, Istanbul Medical Faculty, Istanbul University
[3]Department of Neurology, Istanbul Medical Faculty, Istanbul University
[4]Private practice, Istanbul, Turkey

Purpose:
Inhibitory killer-cell immunoglobulin-like receptors (KIR), as well as programmed cell death protein-1 (PD-1) expressing CD8+T cells play regulatory roles through inhibition of T cells. This study examines CD8+T cells in MG to evaluate their potential roles in controlling disease development.
Material-Methods:
AChR (+) MG patients (n= 29) and age- and sex-matched healthy controls (HCs, n= 24) were enrolled. Peripheral blood mononuclear cells (PBMCs) were stained with anti-CD3, CD8, PD-1, KIR (KIR2DL1, KIR2DL5, KIR2DL2/L3, KIR3DL2, KIR3DL1), CD28, TIGIT, CD45RA, and CCR7 antibodies and analyzed.
Results:
Higher proportion of CD8+T cells were carrying KIR molecules in MG patients (10.3%) compared to HCs (4.7%, p= 0.01). Among the five KIR subtypes, KIR2DL2/L3 and KIR3DL1 were the most frequent variants in both groups. As shown for CD8+ regulatory T cell phenotype before, KIR+TIGIT+ (9.1 vs. 4.4%, p= 0.01) and KIR+CD28- (10.8 vs. 5.0%, p= 0.01) cells were significantly increased in MG patients compared to HCs. CD8+ T cell subset expressing PD-1 did not reveal any difference between MG patients and HCs (19.3% vs. 24.2%). Among the CD8+T cells, the central memory (CM) (CCR7+CD45RA-) population of was significantly higher in MG patients than in HCs (8.1% vs. 3.1%, p= 0.0009).
Conclusion:
The increase of recently identified regulatory KIR+ CD8+T cells in MG suggest a functional effect of these cells in this disease, as shown in some other autoimmune diseases. Moreover, these increased cells have also shared the KIR+TIGIT+ and KIR+CD28- phenotype in CD8+T cells in this autoimmune disease, supporting their possible inhibitory features.

Keywords: Immune regulation, CD8+ T cells, MG

[PP-039]

Investigation of mitochondrial membrane potential and IL-10 secretion of mouse peritoneal B1 cells upon TLR stimulation

Sudenur Arsoy, Mehmet Karacay, Zeynep Nur Senturk, Ayca Sayi Yazgan
Department of Molecular Biology and Genetics, Istanbul Technical University, Istanbul, Turkey

Background: B1 cells are tissue-resident and innate-like cells that are primarily located in the peritoneal cavity. In the literature, mitochondrial membrane potential (MMP) of mice peritoneal B-1a cells was significantly increased compared to peritoneal B2 cells by using tetramethylrhodamine (TMRM) in an autophagy-dependent manner. However, the effect of Toll-like receptor (TLR) ligands such as PAM3CSK4, LPS, and Helicobacter felis (H. felis) on the MMP and IL-10 secretion levels of mice B1 cells is unknown. Our study aims to assess the effect of different TLR ligands (Lipopolysaccharides (LPS), PAM3CSK4, and H.felis) on the MMP and IL-10 secretion levels of B1 cells.
Materials-Methods: Peritoneal cavity cells were isolated from IL-10-GFP reporter (VertX IL10egfp) mice by peritoneal lavage. These cells were stimulated with TLR4-ligand LPS (5µg/ml), TLR2-ligand PAM3CSK4 (2,5 µg/ml) and H. felis (10µg/ml) for 72h. B1 cells were characterized by CD19 and CD23 surface marker stainings and Mitoview 633 was used to quantify MMP. Both MMP and IL-10 secretion were analyzed by flow cytometry. IL-10 secretion levels were further investigated by IL-10 ELISA.

Results: Our results showed that different TLR ligands, LPS, PAM3CSK4, and H.felis, significantly increased the IL-10 secretion levels and MMP of mouse peritoneal-derived B1 cells compared to the unstimulated cells at 72h. The IL-10 ELISA results validated the findings obtained from flow cytometry.

Conclusion: These results suggest that in vitro stimulation of mouse peritoneal cells with TLR ligands (PAM3CSK4, LPS, and H.felis) enhances IL-10 secretion levels and MMP. The elevated IL-10 levels suggest that these cells are likely to acquire regulatory features upon stimulation. Further studies are needed to explore the energy metabolism of B1 cells, particularly B1-a cells.

Acknowledgments: This study was supported by Health Institutes of Türkiye (TUSEB), (Project Number: 32714) and Istanbul Technical University Department of Scientific Research Projects (ITU-BAP) (Project Number: 44621)

Keywords: B1 cells, Peritoneal cavity, IL-10, Mitochondrial membrane potential

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[PP-040]

Neutrophilic activity against Cutibacterium acnes in sarcoidosis patients

Emre Yanardağ[1], Himmet Şirin[2], Muammer Bilir[3], Suzan Çınar[4]
[1]Department of Immunology, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Istanbul, Türkiye; Institute of Graduate Studies in Health Sciences, Istanbul University, Istanbul, Türkiye; Department of Internal Medicine, Istanbul Training and Research Hospital, University of Health Sciences, Istanbul, Türkiye
[2]Department of Immunology, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Istanbul, Türkiye; Institute of Graduate Studies in Health Sciences, Istanbul University, Istanbul, Türkiye
[3]Department of Internal Medicine, Cerrahpasa Faculty of Medicine, Istanbul University-Cerrahpasa, Istanbul, Türkiye
[4]Department of Immunology, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Istanbul, Türkiye

Background: Sarcoidosis is a multisystemic autoimmune related disease that can involve any area of the human body. Several mechanisms may be involved in its etiology like microbial organisms. Cutibacterium acnes (C. acnes), an anaerobic, gram-positive rod bacteria endemic to the skin and mucosal surfaces, has been suggested to be related with etiology. We aimed to investigate the neutrophilic response to C. acnes in sarcoidosis patients.

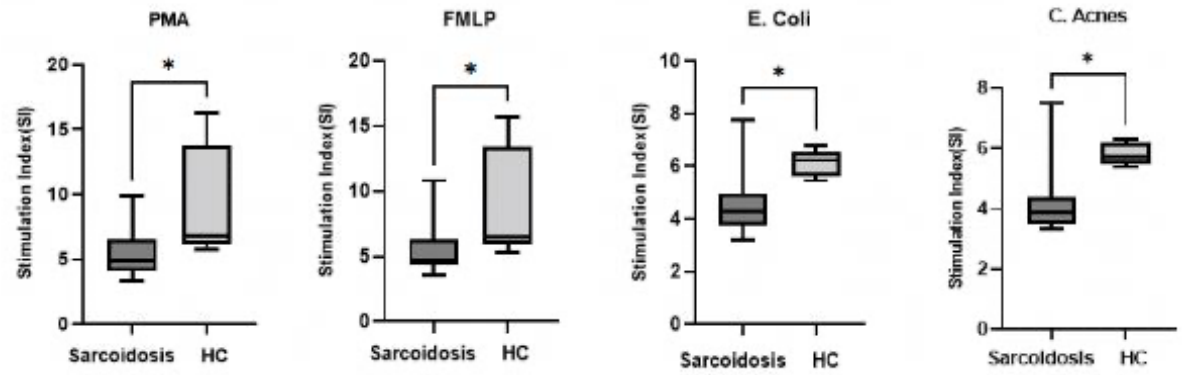
Materials-Methods: Heparinized peripheral venous blood samples of 18 (8 M, 10 F) sarcoidosis patients with mean age of 38 years and 5 (3M, 2F) healthy controls (HC) with mean age of 31 years were taken. To measure oxidative burst activity, PBMC were isolated from whole blood by Ficoll gradient seperation and stimulated with PMA, FMLP, E. coli, and C. acnes seperately. DHR-123 was added as oxidative activity marker. Finally, activity was measured on flow cytometry.

Results: Neutrophil response of sarcoidosis patients against C. acnes stimulation was significantly lower than HC with Stimulation Index (SI) of 3.880 vs 5.720 with p=0.0235. It was of note that response against PMA (4.875 vs 6.790, p=0.151), FMLP (4.680 vs 6.530, p=0.0156) and E.Coli (4.305 vs 6.230, p=0.0243) SI were also lower in sarcoidosis patients compared to HC.

Conclusions: Over the years, relation of C. acnes with etiology and triggering of sarcoidosis has been explored by various studies using bacterial culture, PCR, In Situ Hybridization, immunohistochemistry and other methods and found to be positively correlated in many of these studies. We found that oxidative burst response of neutrophils against C. acnes was weaker in sarcoidosis patients compared to healthy individuals. This suggests either neutrophil exhaustion or C. acnes buildup due to lowered bacterial clearance, and chronic stimulation caused by this, may play a role in development of sarcoidosis but further studies are required to better understand these findings.

Keywords: sarcoidosis, cutibacterium acnes, C. acnes, neutrophil, oxidative burst, autoimmunity

Stimulation Index of Sarcoidosis Patients vs Healthy Controls



[PP-041]

Disease Subtypes in Myasthenia Gravis and T Cell Related Cytokines

Merve Çebi[1], Arman Çakar[2], Hacer Durmuş[2], Yeşim Parman[2], Güher Saruhan Direskeneli[3]
[1]Department of Physiology, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey,
*Current adress: Department of Medical Biology, Medical Faculty, Recep Tayyip Erdoğan University, Rize, Turkey
[2]Department of Neurology, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey
[3]Department of Physiology, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey

Myasthenia gravis (MG) is an autoimmune disorder characterized by autoantibody-mediated impairment of neuromuscular transmission with diverse mechanisms in disease subtypes. Targets of the autoimmune response are mainly acetylcholine receptors (AChR) and muscle-specific kinase (MuSK). The disease exhibits heterogeneity based on autoantibody specificity, disease onset, and the presence of thymoma. T cells play a key role in MG pathogenesis through cytokine-mediated immune regulation. However, the cytokine profile in different MG subgroups remains unclear. This study aimed to evaluate T cell related cytokines in MG patients covering different disease subtypes. AChR-MG patients were stratified into early-onset (EOMG, n=23) and late-onset (LOMG, n=23) subgroups. Additionally, MuSK-MG (n=18), and thymoma-associated MG (TAMG) (n=25), all of whom were not receiving immunosuppressive therapy, along with healthy controls (HC, n=24) were included. Serum cytokine levels were measured using a cytometric bead-based assay (LegendPlex) and compared between disease subgroups from an extended cohort.
Serum levels of IL-4, IL-9, IL-13, IL-17A and IFN-γ were increased in both MuSK-MG and EOMG groups compared with HC. In the MuSK-MG group IL-6, whereas in EOMG IL-2, TNF-α, IL-17F and IL-22 were higher than HC. EOMG patients also showed higher levels of IL-4, IL-9, IL-17F and IFN-γ compared to the LOMG group. Only IL-17A was elevated in TAMG as in MuSK-MG and EOMG groups compared with HC.
Serum cytokine profiles vary across MG subgroups, with prominent role of IL-17A elevated in many indicating its key role in MG pathogenesis. The results in EOMG and MuSK-MG indicate more active immune responses, while LOMG and TAMG, consisting of older patients, exhibit weaker responses, highlighting the impact of aging on immune activation in MG.
This study is supported by Tübitak (116S317) and Istanbul University Research Fund (363804).

Keywords: Myasthenia gravis, Cytokines, IL-17, AChR-MG, MuSK-MG

[PP-042]

Experience of Milk Oral Immunotherapy with and without Omalizumab

Mehmet Tarik Cilingiroglu[1], Cemre Naz Bülbüloglu[1], Gizem Kuruoglu[2], Elif Yaycı[2], Gamze Yaylak[3], Betül Buyuktiryaki[3], Cansın Sackesen[3]
[1]Koç University Graduate School of Health Sciences,Koç University's Research Center for Translational Medicine
[2]Koç University School of Medicine
[3]Koç University School of Medicine, Division of Pediatric Allergy and Immunology

Objective: Our objective was to evaluate the impact of Omalizumab (OMA) on the safety and effectiveness of oral immunotherapy (OIT) for cow's milk allergy and focusing on its role during OIT.

Methods: Twenty-one patients with IgE-mediated CMPA who underwent OIT were included. OMA has started eight weeks before OIT in patients whose parents gave consent for using the drug. Adverse events and allergic reactions were recorded during the treatment and compared patients who receiving OMA or not.

Results: Among the 21 children, 18 showed full adherence to milk OIT. Of the 18 children, 11 were male with a median age of 8.8 years (interquartile range-IQR 5.0-12.6). Median (IQR) total IgE, milk and casein sIgE levels were 676 (275-1076) IU/L, 100 (59-100) kU/L and 99.3 (36-100) kU/L, respectively. Six children completed OIT, and all of them received Omalizumab. In this group, 5 children reached 180-200 ml of pasteurized milk, while 1 child could only tolerate 40 ml due to taste issues and anaphylaxis with 35 ml of milk. Twelve patients are continuing milk OIT, and 4 of them receive Omalizumab. In this group, 1 child who does not receive Omalizumab could only tolerate 2.5 ml due to recurrent anaphylactic reactions. Eleven children still continue to increase the dosage of milk; 4 of them who have received Omalizumab, had no any allergic reactions so far. Seven of them who have not received Omalizumab. Of these seven children 4 of them experienced 1 episodes of allergic reactions and 5 episodes of anaphylaxis. As a report of comorbidity during OIT, 1 case of lactose intolerance and 1 case of GER were observed.

Conclusion: Omalizumab reduces adverse reactions during OIT. Only one child had anaphylaxis with OMA, while five had nine episodes without it during escalation. This suggests Omalizumab enhances safety in OIT escalation.

Keywords: Oral immunotherapy, omalizumab, cow's milk allergy

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[PP-043]

Elucidating T and B cell subsets in pediatric eosinophilic esophagitis patients in the Turkish population

Ceren Buyuk[1], Zeynep Ece Yildiz[1], Muhammed Yuksel[2], Cıgdem Arikan[2], Dilek Guller[3], Ayca Sayi Yazgan[1]
[1]Department of Molecular Biology and Genetics, Istanbul Technical University, Istanbul, Turkey
[2]Koc University School of Medicine, Pediatric Gastroenterology and Hepatology, Organ Transplantation Center, Koc University Research Center for Translational Medicine (KUTTAM), Istanbul, Turkey
[3]Department of Pediatrics, Pediatric Gastroenterology, Demiroglu Bilim University, İstanbul, Turkey

Background / Aim: Eosinophilic esophagitis (EoE) is a chronic allergic inflammatory condition of the esophagus that is triggered by specific food and/or environmental allergens. It is characterized by T helper 2 (Th2)-driven immune response. The immune response observed in EoE differs between adults and pediatric patients and elevated circulating T regulatory (Treg) cell levels in pediatric patients is one such difference. There is not enough study that covers the EoE-related immune response seen in Turkish pediatric EoE patients. In this study, we investigated circulating T and B cell subsets, along with FGL2 and GATA3 mRNA expressions, in Turkish pediatric EoE patients compared to non-EoE controls.
Materials-Methods: Patients in this study were aged between 2-17 and were confirmed to be EoE by both clinical data and eosinophil counts within the tissue. We isolated peripheral blood mononuclear cells (PBMCs) to perform flow cytometry stainings to analyze T and B cell subsets. For intracellular and intranuclear stainings, we stimulated cells with phorbol myristate acetate (PMA)/Ionomycin with Brefeldin A for 5 hours. We synthesized cDNA from distal esophagus biopsies to assess mRNA expressions by qRT-PCR. Data were analyzed using FlowJo 10.8.1 Software and GraphPad Prism 8.0.
Results: Our preliminary data showed that TIGIT+ T helper (Th) cells were significantly higher in peripheral blood of pediatric EoE patients compared to their non-EoE controls. FGL2 and GATA3 mRNA expressions did not show any difference between two groups.
Conclusion: Our data suggests that the levels of certain T and B cell subsets differ between pediatric EoE and control groups, yet patient numbers should be increased.
Acknowledgments: This project is funded by Istanbul Technical University, Department of General Research Projects (ITU-GAP) (Project ID: 43425) Scientific and Technological Research Council of Turkey (TUBITAK-1002B) (Project ID: 124S482).

Keywords: EoE, pediatric, T cell, B cell

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[PP-044]

Anti-PLA2R IgG ELISA: experience from a tertiary center

Melis Gövdeli[1], Şafak Mirioğlu[2], Ayşe Engin[3], Fatma Betül Öktelik[1], Ayşenur Kökoğlu[1], Halil Yazıcı[4], Günnur Deniz[3], Suzan Çınar[3]
[1]Graduate School of Health Sciences, Istanbul University, Istanbul, Turkiye; Department of Immunology, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Istanbul, Turkiye
[2]Graduate School of Health Sciences, Istanbul University, Istanbul, Turkiye; Division of Nephrology, Department of Internal Medicine, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkiye
[3]Department of Immunology, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Istanbul, Turkiye
[4]Division of Nephrology, Department of Internal Medicine, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkiye

Introduction: Antibodies against M-type phospholipase A2 receptor (PLA2R) are used for diagnosis and monitoring of disease activity in membranous nephropathy which is the leading cause of nephrotic syndrome in adults. Lack of data on anti-PLA2R IgG levels in our population is apparent. Therefore, we aim to present 7 years of experience with anti-PLA2R IgG testing at our institution, providing insights into its clinical utility and epidemiological significance.

Methods: Since 2019, anti-PLA2R IgG testing has been implemented in our institution for diagnostic or immunomonitoring purposes. For this study, we retrospectively collected data on anti-PLA2R IgG test results obtained by ELISA (EUROIMMUN) from patient sera. Results >20 RU/ml were considered as positive whereas 14-20 RU/ml was defined as the grey zone/borderline and <14 RU/ml as negative. Demographic characteristics including age and sex were analyzed. Patients were divided into the following age groups: 0-20, 20-40, 40-60 and 60+ years. Statistical analyses were performed with SPSS v25.

Results: A total of 857 samples from 647 (55.6% male) patients were tested, with 210 patients undergoing multiple tests. Median age was 54 (IQR: 42-64) years. Of the total cohort, 487 patients (75.3%) had negative results, while 147 (22.7%) tested positive. The grey zone/borderline accounted for 2% (13 patients) of all age groups, with the highest prevalence in 40-60 years (2.6%). The median anti-PLA2R IgG for the entire cohort was 19.9 (2.9-104.2) RU/ml. In age groups of 0-20 and 60+ years, median antibody levels were 8.4 (2.4-356.6) RU/ml and 11.7 (2.7-82.4) RU/ml, respectively. However, in age groups of 20-40 and 40-60 years, median levels were 35.5 (3.5-111.4) RU/ml and 24.5 (3-133.8) RU/ml, respectively.

Conclusions: Borderline results are uncommon in anti-PLA2R IgG ELISA testing. The high percentage of negative results might be related to its use as a screening tool for diagnosis in patients with nephrotic syndrome.

Keywords: ELISA, membranous nephropathy, nephrotic syndrome, PLA2R

[PP-046]

Comparative Evaluation of DEB and H2AX Tests in Combined Immunodeficiencies with Chromosomal Breaks for Diagnostic Algorithm Development

Barış Ulum[1], Canberk Ipşir[2], Anıl Işık[2], Elif Soyak Aytekin[1], Saliha Esenboğa[3], Deniz Nazire Çağdaş Ayvaz[3]
[1]Hacettepe Üniversitesi Tıp Fakültesi, İhsan Doğramacı Çocuk Hastanesi,Ankara
[2]Hacettepe Üniversitesi Çocuk Sağlığı Enstitüsü, Çocuk Temel Bilimleri, Pediyatrik İmmünoloji Anabilim Dalı, Ankara
[3]Hacettepe Üniversitesi Tıp Fakültesi, Çocuk Sağlığı ve Hastalıkları Anabilim Dalı, Pediyatrik İmmünoloji Bilim Dalı,Ankara

Congenital immunodeficiencies (CIDs) are a heterogeneous group of disorders, some of which result from defects in DNA damage repair mechanisms. DNA damage can manifest as single-base changes, single or double-strand breaks, and cross-linking. Chromosomal breaks may arise due to environmental factors such as radiation and reactive oxygen species, or through physiological processes like isotype switching and somatic hypermutation in lymphocytes. Conditions such as ataxia-telangiectasia, Artemis deficiency, and Ligase-4 deficiency lead to immunodeficiency due to impaired DNA repair mechanisms. γ-H2AX phosphorylation (p-H2AX) and diepoxybutadiene (DEB) tests play an important role in detecting DNA breaks, yet their comparative diagnostic value remains unclear.

This study evaluated the diagnostic efficiency of p-H2AX and DEB tests in patients with primary immunodeficiencies exhibiting chromosomal instability. Peripheral blood samples from patients and healthy controls were exposed to ionizing radiation at various doses (0 Gy, 2 Gy, 5 Gy, 8 Gy, 12 Gy, and 18 Gy). p-H2AX expression was assessed through immunofluorescence staining and flow cytometry using H2AX and p-H2AX antibodies. DEB testing was performed to determine chromosomal breakage rates. Statistical analyses were conducted to compare the effectiveness of these tests.

Comparative analysis revealed significant differences in p-H2AX expression levels between patient and control groups. No statistically significant difference was observed at 0 Gy ($p = 0.2660$), whereas a notable increase in p-H2AX expression was detected in patients at 5 Gy ($p = 0.0037$). Immunofluorescence staining supported these findings. These results suggest that H2AX testing is a sensitive biomarker for assessing radiosensitivity in CIDs. Compared to DEB testing, H2AX analysis appears to be a faster and more reliable method for diagnosing chromosomal instability and guiding clinical decision-making in primary immunodeficiency disorders.

Keywords: Chromosomal Instability, Radiosensitivity, Primary Immunodeficiency

Figure 1: Immunofluorescence staining

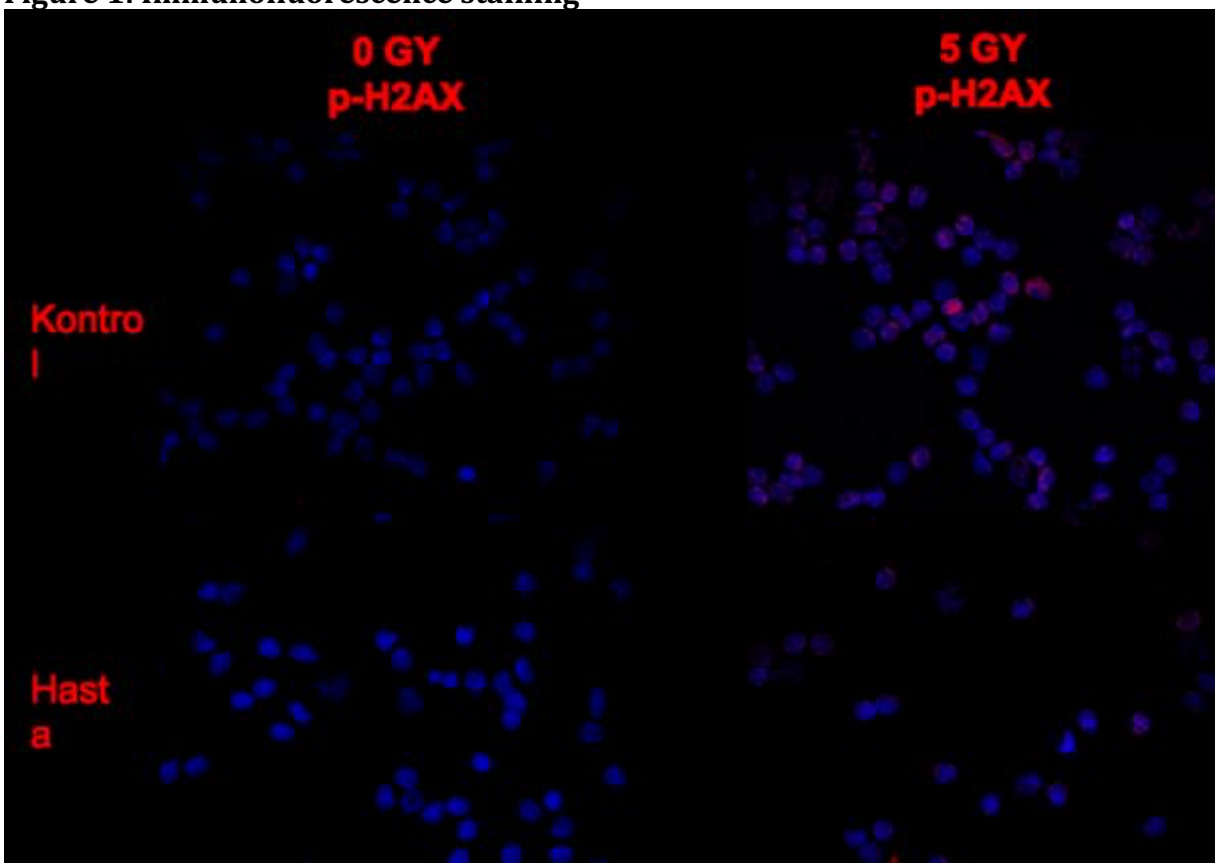

Figure 1: Immunofluorescence staining of p-H2AX in patient and control groups exposed to 0 Gy and 5 Gy radiation. Blue: DAPI, Red: p-H2AX.

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[PP-048]

Evaluation of CXCR5 Expressing Natural Killer Cells in Chronic Lymphocytic Leukemia

Melis Irem Kuyas[1], Fatih Akboga[1], Fehmi Hindilerden[2], Ipek Yonal Hindilerden[3], Emine Gulturk[2], Elif Aksoy[2], Gunnur Deniz[1], Metin Yusuf Gelmez[1]
[1]Istanbul University, Institute of Health Sciences, Istanbul, Turkiye; Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Immunology, Istanbul, Turkiye
[2]Istanbul Dr. Sadi Konuk Training and Research Hospital, Division of Internal Medicine, Department of Hematology, Istanbul, Turkiye
[3]Istanbul University, Istanbul Medical Faculty, Division of Internal Medicine, Department of Hematology, Istanbul, Turkiye

Objective: Chronic lymphocytic leukemia (CLL) is characterized by the accumulation of malignant B cells in lymph nodes, spleen and bone marrow. Natural killer (NK) cells expressing CXCR5 have been shown to migrate to lymph node follicles to lyse tumor/infected cells. Activator receptors, immune-checkpoint molecules, and intracellular levels of IFN-γ, granzyme B, and perforin were analyzed in NK and CXCR5+ NK cells from CLL patients.
Materials-Methods: Peripheral blood mononuclear cells were isolated from 24 CLL patients and 20 healthy controls and stimulated with PMA/Ionomycin for 4 hours. Cells were labeled with anti-CD3, -CD56, -CD16, -CXCR5, -PD-1, -TIGIT, -TIM-3, -LAG-3, NKG2D, -NKp30, -NKp44 and -NKp46 monoclonal antibodies. After fixation/permeabilization, cells were stained with anti-IFN-γ, -perforin and -granzyme B and evaluated by flow cytometer.
Results: In healthy individuals, CXCR5+ NK cells exhibited higher NKp44 and PD-1 expression but lower NKp30, NKp46, NKG2D, TIGIT, IFN-γ, granzyme B, and perforin than CXCR5- NK cells. NK cell percentages were significantly reduced in CLL patients, though NK cell counts did not differ significantly from controls. Malignant CD5+CD19+ B cells positively correlated with CXCR5+ NK cells but negatively correlated with NK cells. Compared to controls, CLL patients exhibited reduced NKG2D, TIGIT, IFN-γ, granzyme B and perforin in NK cells, while CXCR5+ NK cells showed decreased NKp44, TIGIT, Granzyme B, but increased perforin and PD-1.
Conclusions: Since NKp44 and PD-1 are exclusively expressed on activated NK cells, their upregulation in CXCR5+ NK cells suggests enhanced activation. Additionally, reduced perforin in NK cells but increased in CXCR5+ NK cells, along with the positive correlation between malign B cells and CXCR5+ NK cells, indicates that CXCR5+ NK cells may play a key role in CLL pathogenesis.

This project is supported by I.U. BAP (Project no: 40733).

Keywords: Chronic Lymphocytic Leukemia (CLL), Natural Killer (NK) Cells, CXCR5, CXCR5+ NK Cells

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[PP-049]

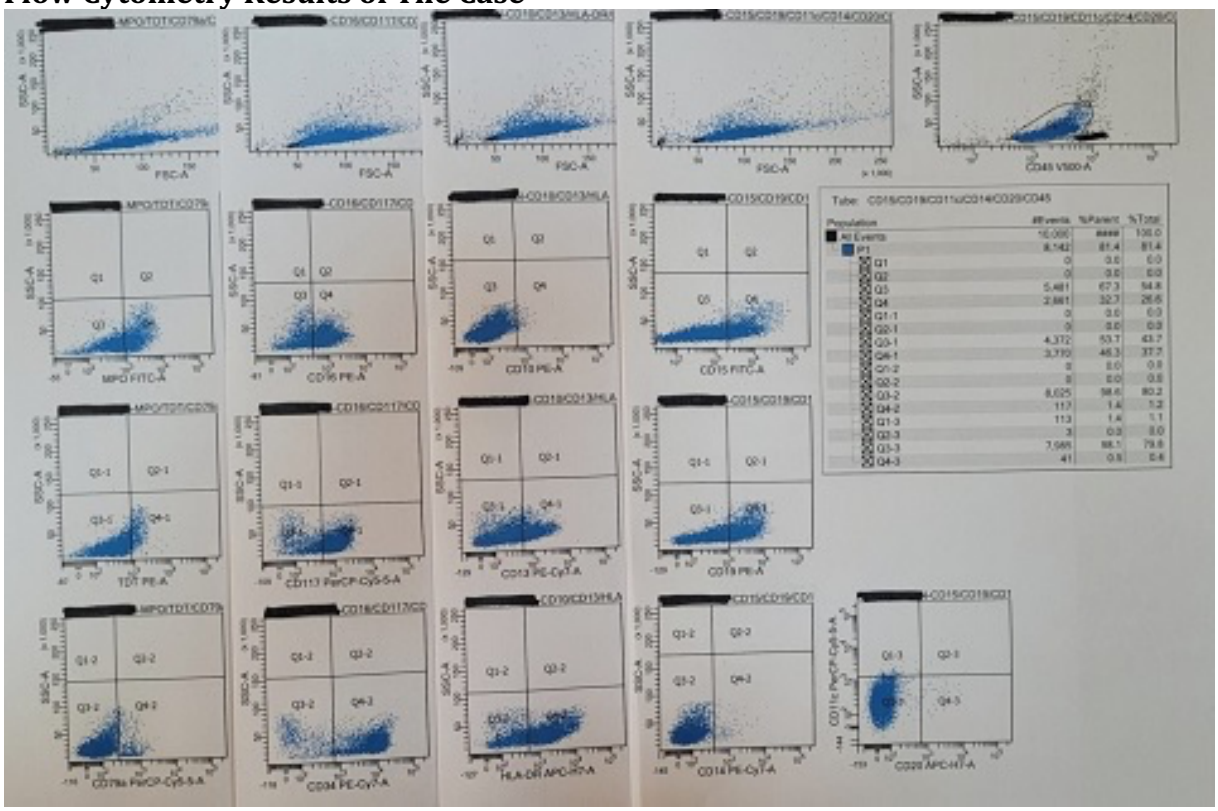
Aberrant CD19 Expression in AML-M0 with t(8;21): Diagnostic Complexity and Therapeutic Challenges

Gülşah Kaygısız[1], İbrahim Ethem Pınar[2], Server Yağcı[3], Fatma Aksoy Khurami[4]
[1]Afyonkarahisar Public Health Laboratory
[2]Isparta City Hospital Hematology Clinic
[3]Isparta Süleyman Demirel University Department of Medical Microbiology
[4]Isparta City Hospital Pathology Clinic

Introduction:
Minimal differentiated acute myeloid leukemia (AML-M0) is a rare subtype constituting 2–3% of AML cases. Immunophenotyping is essential for distinguishing AML-M0 from similar entities. The t(8;21) translocation generally confers a favorable prognosis due to high sensitivity to cytarabine-based treatments. However, aberrant CD19 expression complicates this, posing diagnostic and therapeutic challenges, particularly in minimal residual disease (MRD) monitoring and relapse risk.
Case Presentation:
A 61-year-old female with depression presented with bruising and body pain. Examination revealed ecchymoses, pallor, and no organomegaly. Laboratory results showed hemoglobin 7.3 g/dL, WBC 51.74 × 10^3/μL, platelets 23 × 10^3/μL, and elevated LDH. Peripheral smear indicated acute leukemia. Bone marrow revealed 93% hypercellular blasts. Immunophenotyping confirmed MPO, CD13, CD34, and CD117 positivity (myeloid markers) and aberrant CD19 expression (46.3%). Cytogenetics showed t(8;21) and trisomy 8 in 87% of cells, classifying the case as AML under ENL2022 and WHO criteria.
Discussion:
Although t(8;21) predicts better outcomes, CD19 positivity introduces a mixed phenotype, complicating MRD monitoring and increasing relapse risk. High-dose Ara-C (HDAC) is effective for t(8;21)-positive AML, but CD19 expression challenges response durability. While CAR-T cells and anti-CD19 antibodies are successful in B-cell malignancies, their role in AML is unclear. In this case, the use of flow cytometry for immunotyping was crucial for early identification of the phenotype, allowing prompt diagnostic classification and management. Future strategies must integrate myeloid and lymphoid-directed therapies. Personalized approaches, incorporating genetic and immunophenotypic insights are crucial for optimal outcomes.
Conclusion:
This case highlights the complexities of AML-M0 with t(8;21) and CD19 aberrancy. Standard HDAC protocols are effective, but additional strategies addressing mixed phenotypes are needed. Understanding aberrant antigen expression and advancing targeted therapies can improve outcomes for this challenging subgroup.

Keywords: Aberrant CD19 Expression, AML-M0, mixed phenotypes, targeted therapies, t(8;21)

Flow Cytometry Results of The Case



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[PP-050]

Inhibition of IFN-γ-Induced MIF Signaling in Breast Cancer Cell Lines

Ayşenur Kökoğlu[1], Ali Abdi[1], Esin Çetin[1], Suhendan Ekmekcioğlu[2], Günnur Deniz[1]
[1]Department of Immunology, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Istanbul, Turkey
[2]Department of Melanoma Medical Oncology - Research, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX

Objective: Macrophage migration inhibitory factor(MIF) is overexpressed in breast cancer and is associated with metastasis and poor prognosis. Upon binding to its receptor CD74,MIF initiates signaling pathways involved in cancer progression, including COX-2 and PGE2 pathways. This study investigates the effect of IFN-γ on MHC-II-mediated and MIF/CD74/CXCR signaling in cancer cell survival.
Material-Methods: MCF-10Aand MDA-MB-231 cells were treated with COX-2 inhibitor celecoxib, PGE2 synthetase inhibitor MF63,and MIF antagonist ISO-1 for 48 hours, with or without IFN-γ. Flow cytometry was used to analyze CD74, CD44, HLA-DR, CXCR2, CXCR4, CXCR7, PD-L1, and PD-L2 expression. ELISA was performed to quantify soluble MIF, CD74, and PGE2, and cytokine levels were assessed using LegendPlex.
Results: IFN-γ upregulated CD74, HLA-DR, PD-L1, and PD-L2 in both cell lines. Celecoxib and ISO-1 suppressed CD74, while MF63 inhibited IFN-γ induced CD74 expression in MDA-MB-231 cells. IFN-γ also modulated cytokine secretion, increasing MCP-1 and IL-6 in both cell lines. However, in MCF-10A cells, IFN-γ decreased soluble IL-1β,IL-8, and IL-18 levels, suggesting a suppression of pro-inflammatory signaling. In contrast, these cytokines were upregulated in MDA-MB-231 cells, indicating a tumor-promoting inflammatory response.
Discussion: IFN-γ may promote tumor progression by upregulating CD74 and immune checkpoint molecules. The suppression of CD74 by celecoxib, MF63, and ISO-1 highlights their therapeutic potential in targeting the MIF/CD74 pathway. IFN-γ induced MCP-1 and IL-6 elevation may create a pro-inflammatory yet immunosuppressive tumor microenvironment. MCP-1 recruits macrophages, potentially driving M2 polarization, while IL-6 enhances tumor proliferation and metastasis. The reduction of IL-1β,IL-8, and IL-18 in MCF-10A cells suggests suppressed inflammation in non-tumorigenic cells, whereas their increase in MDA-MB-231 cells may foster a tumor-supportive niche. These findings suggest that IFN-γ exerts dual effects in the tumor microenvironment, acting as either tumor-promoting or suppressive depending on context.

This project was funded by the Research Fund of Istanbul University (BAP-TSA-2023-39677 and TSA-2022-39189).

Keywords: Breast cancer, TNBC, IFN-γ, cytokine

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[PP-051]

Evaluation of the Anti-Inflammatory and Anticancer Potential of Novel Salicylic Acid Derivatives on Malignant Melanoma *In Vitro*

Başak Aru[1], Tuğçe Özyazıcı[2], Furkan Aydın[1], Ayşe Nur Çaldıran[1], Meriç Köksal Akkoç[3], Gülderen Yanıkkaya Demirel[1]
[1]Immunology Department, Faculty of Medicine, Yeditepe University
[2]Department of Pharmaceutical Chemistry, Hamidiye Faculty of Pharmacy, University of Health Sciences
[3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Yeditepe University

While nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to reduce inflammation by inhibiting cyclooxygenases (COX), recent data underline their potential for cancer treatment. This study aims to evaluate the anticancer potential of four non-commercial salicylic acid derivatives on cutaneous malignant melanoma, the most aggressive and lethal form of skin cancer, accounting for 80% of all skin cancer-related deaths.
The anti-inflammatory effects of the compounds, **5e**, **5h**, **4-OH 5e**, and **4-OH 5h** were evaluated at the gene expression level on RAW 264.7 cell line, where cellular inflammation was induced with LPS and then cells were treated with the compounds. For evaluating compounds' anticancer properties, relative viability percentages upon treatments were evaluated with MTS assay, and IC50 values for each compound was calculated for 24 and 48 hours on four malignant melanoma and one healthy keratinocyte cell line. The effects of the compounds on intracellular ROS levels and apoptosis were evaluated using DHR123 and Annexin V/propidium iodide stainings, respectively. Our results showed that all compounds successfully decreased iNOS and COX2 gene expression levels on LPS-treated RAW 264.7 cells, although compounds with hydroxyl group at ortho positions were superior in terms of suppressing inflammatory mediators IL-1β, IL-6 and TNFα. In malignant melanoma cell lines, the lowest IC50 values were calculated at 48 hours, and a significant decrease in intracellular ROS levels was observed at both 24 and 48 hours compared to the LPS group when cells were treated with compounds at their respective IC50 doses. Annexin V/propidium iodide staining revealed that the compounds induce apoptosis but not necrosis. Altogether, these results underscore the anticancer potential of these compounds and warrant further studies that will focus on investigating the effects of the compounds on autophagy and pyroptosis on malign melanoma.

This study was supported by TÜBİTAK – 1001 (Project No: 123Z047).

Keywords: Malignant Melanoma, Non steroidal Anti Inflammatory Drugs, Cyclooxygenases, Apoptosis

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[PP-052]

Generation of nanobody based chimeric antigen receptor constructs against CD19 positive B cell lymphomas

Büşra Şimşek[1], Alp Ertunga Eyüpoğlu[3], Fatma Sude Albayrak[2], Elif Çelik[2], Tolga Sütlü[2], Batu Erman[2]
[1]Molecular Biology and Genetics, Boğaziçi University, Istanbul, Turkey
[2]Molecular Biology and Genetics, Acıbadem University, Istanbul, Turkey
[3]Molecular Biology and Genetics, Boğaziçi University, Istanbul, Turkey; Molecular Biology and Genetics, Acıbadem University, Istanbul, Turkey

Thanks to its therapeutic potential, CAR-T cell therapy became one of the most promising immunotherapy techniques with the approval of four different CD19 targeting CAR-T cell products by the FDA for the treatment of refractory and relapsed B-cell malignancies. However, all these therapies were developed using scFv as a ligand binding domain, shown to decrease therapeutic efficacy due to its large size, high level of immunogenicity and propensity for self-aggregation on the membrane leading to ligand independent activation. Nanobodies are promising alternatives to scFv, based on their comparable binding potential, higher stability and solubility, and lower immunogenicity. In this study, we aimed to develop novel nanobody-based anti-CD19 CAR constructs eventually to be used in the treatment of B-cell malignancies. First, we designed a nanobody-based CAR construct using the second-generation CAR backbone, achieving comparable surface expression to scFv-based CAR. Next, we tested more than ten anti-CD19 nanobodies, selected from an immune library using phage display, evaluating their binding and activation potentials. These nanobody-CAR constructs were ranked according to affinity and tested in Jurkat-Raji cell co-culture experiments by assessing CD69 activation. In summary, we obtained three CD19 specific nanobody-CAR candidates with nanomolar affinities that were successfully expressed both on the Jurkat and primary T cell surface and activated these cells upon specific CD19 binding on target B lymphocytes. Epitope binning showed that one of these nanobodies recognizes an overlapping epitope to the standard anti-CD19 ScFv CAR's while two bind to non-overlapping epitopes. In vivo testing is ongoing in NSG mice with Raji cell xenografts.

Keywords: B-cell malignancies, cancer immunotherapy, chimeric antigen receptor T-cell (CAR-T) therapy, human CD19, nanobody

[PP-053]

Detection of Natural Killer Cell Levels in Bone Marrow of Childhood T- ALL Cases by Flow Cytometry

Fatih Akboga, Naser Seyyidi Shouha, Metin Yusuf Gelmez, Umut Can Kucuksezer, Gunnur Deniz, Suzan Adin Cinar
Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Immunology, Istanbul, Türkiye

INTRODUCTION
T- (acute lymphoblastic leukemia) ALL is an aggressive leukemia with molecular and immunophenotypic diversity, its prognosis primarily relies on minimal residual disease (MRD) assessment by Flow cytometry due to limited independent risk markers. Relapse or refractory disease occurs in 15% of children and 30% of adults. Enhancing risk stratification is crucial for identifying high-risk patients who may benefit from targeted therapy. Natural killer (NK) cells are key effectors of innate immunity, serving as a first line of defense against cancer. In this study, we aimed to determine NK cell levels at diagnosis in childhood T-ALL and investigate their potential correlation with MRD, providing insights into their prognostic significance.
METHOD
This study analyzed NK cell levels in bone marrow samples from T-ALL patients (n=150, M=115/F=35) collected between 2012 and 2022. Flow cytometry was used to assess CD3-CD56+ NK cell expression and MRD levels in leukemic cells. Patients were grouped based on age, gender, MRD positivity/negativity, and statistical comparisons were conducted using Mann-Whitney U test and regression analysis. Correlation analysis between NK cell levels and MRD status was performed using Spearman's correlation test. All statistical analyses were conducted using Graphpad Prism 9.2 and R software.
RESULTS
In T-ALL patients, no significant differences were observed in NK levels based on age, gender, white blood cell count at diagnosis, or MRD status on day15. Furthermore, correlation and regression analyses between NK levels at diagnosis and day15 MRD showed no significant association.
DISCUSSION
Mizia-Malarz et al. proposed that NK cell presence in the bone marrow at diagnosis may serve as a prognostic factor in children with ALL. However, in our study, correlation, regression, and ROC analyses in children with T-ALL did not support this association. These findings suggest that NK levels alone may not be a reliable prognostic indicator in this subset of patients.

Keywords: T-ALL, MRD, NK Cells

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[PP-054]

The Effect of 4T1 Conditioned Medium on Splenic Memory T Cell Subpopulations

Batuhan Yağcioğlu[1], Onur Etgü[1], Haluk Barbaros Oral[2], Diğdem Yöyen Ermıs[2]
[1]Bursa Uludag University Institute of Health Sciences, Department of Medicine-Immunology, Bursa,Turkey, Bursa Uludag University Faculty of Medicine, Experimental Animal Breeding and Research Unit (Denhab), Bursa,Turkey
[2]Bursa Uludag University Faculty of Medicine, Department of Immunology, Bursa, Turkey, Bursa Uludag University Faculty of Medicine, Experimental Animal Breeding and Research Unit (Denhab), Bursa,Turkey

Objective:
Cancer is characterized by uncontrolled cell proliferation and metastatic potential, with triple-negative breast cancer (TNBC) fostering an immunosuppressive microenvironment. Memory T cells play a crucial role in cancer immunity by ensuring long-term immune surveillance and protection. They are classified into three subsets: central memory T cells (TCM), effector memory T cells (TEM), and tissue-resident memory T cells (TRM). This study investigates the differentiation of memory T cells in response to soluble factors secreted by 4T1 tumor cells.
Materials-Methods:
Splenic T cells were isolated from 6-7-week-old female BALB/c mice and cultured for 8 days in the presence of 4T1-conditioned medium (4T1 CM). Cells were stained with CD3, CD4, CD8, CD44, CD45.2,CD62L,CD69,CD103,KLRG1,CXCR3, and CCR7 antibodies, followed by flow cytometric analysis. Memory T cell subsets were phenotyped as follows all subgroups have CD3+, CD4+ or CD8+, CD44+(hi), CD45.2+(hi) markers: TRM ($CD62L^{-}$,$CD69^{+}$,$CD103^{+}$, $CXCR3^{+}$,$CCR7^{-}$,$KLRG1^{-}$),TCM($CD62L^{+}$, $CD69^{-}$, $CD103^{-}$,$CXCR3^{+}$,$CCR7^{+}$,$KLRG1^{-/+}$), and TEM ($CD62L^{+}$,$CD69^{-}$,$CD103^{-}$, $CXCR3^{-}$, $CCR7^{-}$, $KLRG1^{+}$). Purified subsets (90% purity) were co-cultured with 4T1 cells at an 8:1 ratio for four days. Viability was assessed by PI staining using $CD45^{-}$/$CD3^{-}$ gating.
Results and Conclusion:
Following the purification of memory T cell subsets from splenocytes cultured long-term with 4T1-conditioned medium, cells were co-cultured with 4T1 tumor cells. After incubation, $CD4^{+}$TCM, $CD4^{+}$TEM, $CD8^{+}$TRM, $CD8^{+}$TCM (107%), and $CD8^{+}$TEM were found to support 4T1 cell viability, whereas the $CD4^{+}$TRM subset exhibited a suppressive effect, reducing viability to 52%. These findings suggest that one of CD4+TRM cells subgroup expanded in response to exhibit significant cytotoxic effects on 4T1 tumor cells. Understanding the interactions between the tumor microenvironment and memory T cells could inform novel immunotherapeutic approaches for TNBC.
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Keywords: Memory T cell, Triple-negative breast cancer (TNBC), 4T1 Cell Line

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[PP-055]

Metabolic stress induces LAG3 upregulation on Th1 cells

Sakine Ulusoy, Neşe Ünver, Güneş Esendağlı
Hacettepe University Cancer Institute, Department of Basic Oncology, Ankara, Türkiye

T cell exhaustion has been associated with continuous stimulation; however, metabolic changes regulated by the extracellular microenvironment are also crucial. This study aims to investigate the immunophenotypic and functional effects of cancer cell-derived metabolites on Th1 cell functions. Peripheral blood mononuclear cells (PBMCs) were isolated from healthy donors using Ficoll gradient separation, and CD4+ T cells were enriched via MACS. Naïve CD45RA+CCR7+ T cells were isolated by FACS and co-cultured at different ratios with THP-1 leukemia cells, monocytes, or monocyte-derived dendritic cells. Additionally, co-cultures were established using THP-1 conditioned medium. Proliferation kinetics was assessed by CFSE dilution, and cell viability was determined using Annexin V-PI staining. Expression kinetics of inhibitory receptors PD-1, CTLA-4, LAG3, and TIM-3 were analyzed by flow cytometry. Increasing THP-1 cell ratios in the co-cultures decreased the CD4+ T cell proliferation and viability after 120 hours, with upregulation of inhibitory receptors, particularly LAG3. In monocyte co-cultures and αCD3/28-stimulated Th cell cultures, LAG3 levels were significantly higher when refreshed with THP-1 conditioned medium compared to fresh medium. Similarly, co-cultures established with conditioned medium exhibited higher LAG3 expression than those maintained with fresh medium. These preliminary findings suggest that, in addition to antigenic stimulation and co-stimulation, metabolic stress may play a functional role in LAG3-mediated T cell exhaustion.
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Keywords: T cell activation, costimulation, T cell exhaustion, metabolic stress

[PP-056]

DANCR as a Prognostic Marker and Therapeutic Target in Melanoma

Ege Amirak, Fatih Sezer, H. Atakan Ekiz
Molecular Biology and Genetics, Izmir Institute of Technology, Izmir, Turkey

Introduction: Long noncoding RNAs (lncRNAs) play key roles in cancer progression and immune modulation. Among them, differentiation antagonizing non-protein coding RNA (DANCR) has been linked to tumor development, yet its role in melanoma aggressiveness and immune evasion remains poorly understood. To clarify this, we analyzed bulk RNA sequencing data from The Cancer Genome Atlas Skin Cutaneous Melanoma (TCGA-SKCM) dataset, which revealed DANCR as a negative prognostic marker, with elevated expression correlating with reduced survival.

Methods: To determine how DANCR expression is distributed within the tumor microenvironment (TME), we reanalyzed a previously published single-cell RNA sequencing (scRNA-seq) dataset. To assess the functional consequences of DANCR modulation, we performed CRISPR-dCas9 activation/inhibition and shRNA knockdown experiments in melanoma cells. The effects of these manipulations were examined through proliferation, viability, and apoptosis assays in response to oncogenic signaling inhibitors. Additionally, live-cell imaging, migration, and invasion assays were conducted to evaluate tumor dynamics. Finally, to explore the clinical significance of DANCR expression, we performed receiver operating characteristic (ROC) analysis to assess its predictive value for immune checkpoint blockade (ICB) therapy response.

Results: DANCR expression was primarily detected in melanoma cells, indicating a tumor-intrinsic role in disease progression. Kaplan-Meier survival analysis demonstrated that high DANCR levels correlated with poor prognosis, particularly in PTEN-high cases, suggesting a PI3K pathway association. Functional assays further showed that DANCR overexpression enhances mesenchymal and angiogenic markers, reinforcing its role in tumor aggressiveness. Moreover, patients with lower DANCR expression exhibited better responses to anti-PD-1 therapy, while ROC analysis revealed that DANCR predicts ICB resistance in nearly 70% of non-responders.

Conclusion: Collectively, these findings suggest that DANCR promotes melanoma progression by driving tumor aggressiveness and fostering an immune-evasive state. Its strong correlation with poor survival and ICB resistance highlights its potential as both a prognostic biomarker and a therapeutic target in melanoma.

Keywords: Long noncoding RNAs, Melanoma, Tumor progression, Immune Evasion, Therapy Resistance, Prognostic Biomarker

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[PP-057]

Engineering Mesothelin-Targeted CAR-NK Cells for Enhanced Cancer Immunotherapy

Zeynep Sevinç[1], Aylin Mumcuoğlu[1], Betül Çakıcı[1], Serra Özarı[2], Tolga Sütlü[1]
[1]Acıbadem Mehmet Ali Aydınlar University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics, Istanbul/Turkey
[2]Boğaziçi University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics, Istanbul/Turkey

Objective: Chimeric Antigen Receptor (CAR)-engineered immune cells have revolutionized cancer immunotherapy by enabling antigen-specific tumor targeting. While CAR-T cell therapy has shown remarkable success in hematologic malignancies, CAR-NK cells offer a safer approach that might be applicable in the allogeneic setting. Mesothelin is a cell surface molecule overexpressed in several cancers (e.g., mesothelioma, cervical) while exhibiting limited expression in normal tissues, making it a viable target for CAR-based therapies. However, mesothelin shedding with proteases decreases antigen availability, limiting CAR engagement and activation. Liu et al. (2022) identified the major protease sites in mesothelin (MSLN) and developed a monoclonal antibody (mAb) called 15B6 that binds near the cell membrane, effectively inhibiting MSLN shedding. This study aims to develop and evaluate second-generation CAR-NK cells targeting mesothelin with a 15B6-derived ScFv to counteract antigen shedding and improve therapeutic efficacy against mesothelin-positive tumor cells.
Methods: Lentiviral vectors encoding a second-generation (15B6-28.z) CAR construct were produced via transient transfection of HEK293FT cells. Infectious particles containing supernatant was transduced into NK-92 cells. NK-92 cells were genetically modified with this vector and selected using puromycin resistance. Resulting CAR+ NK-92 cells were assessed for their cytotoxic potential through degranulation and cytokine secretion upon co-culture with mesothelin-expressing and mesothelin-negative target cells.
Result: Our results show that second-generation 15B6-28.z CAR-modified NK92 cells demonstrated high degranulation and cytokine secretion activity upon engagement with MSLN-positive K562 and HEK cells compared to WT and empty vector transduced NK-92 cells.
Conclusion: Our findings suggest that mesothelin-targeted CAR-NK cells exhibited significantly enhanced antigen-specific recognition, leading to improved cytotoxic activity against MSLN-positive tumor cells in vitro. These results highlight the potential of CAR-NK cell therapy as a promising strategy for treating MSLN-positive cancers and warrant exploration in in vivo settings.

Keywords: Chimeric Antigen Receptor (CAR), Natural Killer (NK) cells, Mesothelin, Cancer Immunotherapy

[PP-058]

Combined Therapy with Zebularine and CpG Oligodeoxynucleotides for *in vitro* Melanoma Treatment

Havva Homak, Banu Bayyurt Kocabas
Middle East Technical University

Cancer immunotherapy is a promising treatment that boosts person own immune system against cancer. However, it is unable to entirely remove cancerous cells. Therefore, it is usually combined with other treatments like chemotherapy or radiotherapy to overcome limitations. Another novel cancer treatment is epigenetic therapy which reverse epigenetic dysregulations in cancer cells and might additionally influence immune cells to eliminate suppressive tumor microenvironment. In our previous study, we showed that combination of CpG oligodeoxynucleotides (ODN), TLR9 agonists, and zebularine, an inhibitor of DNMT1, had immunostimulatory effect on primary murine immune cells without reducing adjuvant efficiency. For this study, we aim to treat B16 melanoma cells with the combination to determine the anti-cancer activity of the therapy. For this purpose, B16-F10 cells were treated with CpG ODN and/or zebularine for 48 hours. Afterwards, cell viability was evaluated, type I IFN secretion was measured using B16-Blue IFN-α/β reporter cells, and PD-L1/2, co-inhibitory molecules, was detected by flow cytometry. RASSF1, a tumor suppressor gene, and ERVL, retroelement gene expression analysis was determined through RT-PCR and normalized to HPRT as housekeeping gene. We observed that cell viability was unaffected by CpG ODN alone, but when combined with zebularine, it significantly decreased B16-F10 melanoma cell viability and upregulated RASSF1 and ERVL expression. However, type I IFN secretion was not induced, and PD-L1/2 levels were not changed. Next, we will encapsulate our novel combination into a pH sensitive liposome for effective drug delivery and test using *in vivo* melanoma mouse model. Additionally, we will use a 5-mC DNA ELISA Kit to evaluate the global DNA methylation in treated B16 cells to observe DNMT suppression.

Keywords: Immunotherapy, Epigenetic inhibitors, TLR9, STING, Melanoma

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[PP-059]

Effect of Magnesium on Myeloid-Derived Suppressor Cells in Triple Negative Breast Cancer Model

Ilayda Memiş[1], Onur Etgü[1], Haluk Barbaros Oral[2], Diğdem Yöyen Ermiş[2]
[1]Department of Immunology, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey;Experimental Animal Breeding and Research Unit, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey;Experimental and Molecular Immunology Laboratory (Experim), Faculty of Medicine, Bursa Uludag University, Bursa, Turkey;Department of Medicine-Immunology, Institute of Health Science, Bursa Uludag University, Bursa, Turkey
[2]Department of Immunology, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey;Experimental Animal Breeding and Research Unit, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey;Experimental and Molecular Immunology Laboratory (Experim), Faculty of Medicine, Bursa Uludag University, Bursa, Turkey

Objective: Breast cancer remains the most prevalent malignancy in women, with triple-negative breast cancer(TNBC) being among the most aggressive subtypes.TNBC exhibits high metastatic potential and limited targeted therapeutic options.The tumor microenvironment is heavily influenced by immunosuppressive myeloid-derived suppressor cells(MDSCs), which impair the anti-tumor immune response.This study investigates the impact of magnesium($MgCl_2$) on MDSCs in a murine 4T1 breast cancer model, aiming to elucidate its potential role in breast cancer treatment.
Materials-Methods: Female BALB/c mice(6-8 weeks old) were inoculated with 4T1 cells into the left mammary fat pad. On day 10,after tumor formation, mice were administered low(30mg/kg) or high(60mg/kg) doses of $MgCl_2$intraperitoneally.On day 25, mice were sacrificed, and blood, spleen, bone marrow, and tumor tissues were collected.Flow cytometry was used to analyze MDSC phenotypes (CD11b,Ly6G,Ly6C).Additionally, T-cell suppressive capacities of G-MDSC and M-MDSC subsets isolated from the spleens of high-dose $MgCl_2$-treated mice (pre and post treatment) were assessed.
Results and Conclusion: Tumor volumes were lower in mice receiving $MgCl_2$ before tumor injection compared to post-injection groups.CD11b expression was highest in the blood of pre-tumor groups, while the highest levels in tumors were observed in the 60mg/kg group.G-MDSCs were elevated in the blood of the 30 mg/kg group but lowest in the spleen of the 60mg/kg group.M-MDSCs were most abundant in the blood and spleen of the pre-tumor 60mg/kg group.Notably, M-MDSCs from the spleens of post-tumor high-dose $MgCl_2$ groups exhibited enhanced T-cell suppressive capacity. $MgCl_2$ modulates MDSC levels in TNBC.Pre-tumor administration of $MgCl_2$ reduced the immunosuppressive capacity of MDSCs,whereas post-tumor administration increased both their suppressive function and frequency.Additionally, survival analysis indicated that post-tumor $MgCl_2$ administration promoted tumor progression,whereas pre-tumor $MgCl_2$ treatment was associated with improved survival outcomes.

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Keywords: 4T1 Cell Line, MDSC, Magnesium Chloride, TNBC, Immune Response

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[PP-060]

Artemisinin Triggers Autophagy-Mediated Cell Death in HEPG2 Cells

Mert Karaca[1], Erkan Ermiş[2], Gözde Arslan[1], Salih Haldun Bal[3], Diğdem Yöyen Ermiş[4], Murat Kıyıcı[5], Haluk Barbaros Oral[4]
[1]Bursa Uludag University, Faculty of Medicine, Department of Immunology, Bursa, Türkiye; Bursa Uludag University, Institute of Health Sciences, Medical-Immunology, Bursa, Türkiye; Bursa Uludag University, Faculty of Medicine, Laboratory Animal Breeding and Research Unit (DENHAB), Bursa, Türkiye
[2]Bursa Uludag University, Faculty of Medicine, Department of Pharmacology, Bursa, Türkiye; Bursa Uludag University, Faculty of Medicine, Laboratory Animal Breeding and Research Unit (DENHAB), Bursa, Türkiye
[3]Bursa Uludag University, Faculty of Medicine, Department of Immunology, Bursa, Türkiye; Bursa Uludag University, Faculty of Medicine Hospital, Dr. Raşit Durusoy Blood Center, Bursa, Türkiye; Bursa Uludag University, Faculty of Medicine, Laboratory Animal Breeding and Research Unit (DENHAB), Bursa, Türkiye
[4]Bursa Uludag University, Faculty of Medicine, Department of Immunology, Bursa, Türkiye; Bursa Uludag University, Faculty of Medicine, Laboratory Animal Breeding and Research Unit (DENHAB), Bursa, Türkiye
[5]Bursa Uludag University, Faculty of Medicine, Department of Internal Medicine, Division of Gastroenterology, Bursa, Türkiye

Objective: Hepatocellular carcinoma (HCC) is one of the most aggressive and fatal tumors worldwide, accounting for 82% of primary liver cancers. It is the third leading cause of cancer-related mortality and has a very low five-year survival rate (<15%) due to late diagnosis and underlying liver dysfunction. The number of patients eligible for curative treatments is limited, and alternative therapies fail to improve patient survival. Therefore, there is a significant need for prognostic markers and promising therapeutic strategies for HCC. Dysregulation of autophagy has been associated with tumorigenesis in various cancers, and its role in HCC is well documented. Artemisinin, a molecule extracted from *Artemisia annua L.*, was first discovered as an antimalarial agent and awarded the 2015 Nobel Prize. It is known for its immunomodulatory properties, G0/G1 cell cycle arrest at appropriate doses, and apoptotic effects in various diseases. Although different aspects of artemisinin have been investigated, its autophagic effects in hepatocellular carcinoma cells remain understudied. This study aims to investigate the effect of artemisinin on autophagy-mediated cell death in the HepG2 hepatocellular carcinoma cell line.

Materials-Methods: To assess autophagy, the pcDNA3-GFP-LC3-RFP-LC3ΔG plasmid was utilized. The plasmid was amplified in E. coli, isolated via midiprep, and subjected to restriction analysis. HEPG2 cells were cultured, and the optimal artemisinin dose was determined using the MTT assay. Plasmid transfection was performed using Lipofectamine 6000, autophagosome formation was evaluated using EVOS imaging, and cell death analysis was conducted via flow cytometry.

Results and Conclusion: Autophagosomes were clearly visualized, and artemisinin significantly enhanced autophagy-mediated cell death. These findings suggest that artemisinin may serve as a potential therapeutic agent for HCC treatment.

Keywords: Hepatocellular carcinoma, HCC, HEPG2, Artemisia Anua, Artemisinin

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[PP-061]

Natural killer cell cytotoxicity diminished by tumor-associated schwann cells in pancreatic cancer

Taner Kasapoglu[1], Sila Sigirli[1], Didem Karakas[1], Engin Ulukaya[2], Ihsan Ekin Demir[3], Guralp Onur Ceyhan[4]
[1]Acibadem Mehmet Ali Aydinlar University, Graduate School of Health Sciences, Medical Biotechnology, Istanbul, Turkey
[2]Istinye University, Department of Clinical Biochemistry, Faculty of Medicine, Istanbul, Türkiye
[3]Technical University of Munich, Department of Surgery, Klinikum rechts der Isar, School of Medicine, Munich, Germany
[4]Acibadem Mehmet Ali Aydinlar University, Department of General Surgery, HPB-Unit, School of Medicine, Istanbul, Türkiye

Introduction
Nerves contribute to pancreatic cancer progression, with Schwann cells mediating communication between cancer cells and nerves. Schwann cells are even present in precancerous lesions and can be reprogrammed by pancreatic cancer cells into a tumor-associated phenotype. Although Natural Killer (NK) cells can eradicate early tumor cells, the tumor microenvironment impairs their activity. In this study, we investigated the effect of tumor-associated Schwann cells on NK cell activity against pancreatic cancer cells *in vitro*.

Methods
Schwann cells were reprogrammed with PANC-1 CM, confirmed by GFAP expression via Western blot. CM from Schwann and tumor-associated Schwann cells was applied to NK-92 cells. Calcein-AM labelled PANC-1 cells co-cultured with NK-92 cells for 4 hours, and NK-92 cytotoxicity analyzed. IFN-γ secretion, a marker of cytolytic activity, was measured by ELISA. NK-92 infiltration into PANC-1/Schwann spheroids was assessed with CellTracker dyes.

Results
GFAP expression in Schwann cells treated with PANC-1 CM doubled compared to controls. Schwann cell and tumor-associated Schwann cell CM reduced NK-92 cytotoxicity and decreased IFN-γ secretion. Additionally, Schwann cells in pancreatic cancer spheroids modulated NK-92 infiltration.

Summary
In summary, tumor-associated Schwann cells suppressed NK-92 cytotoxicity via secreted molecules, however the specific factors remain unknown. Further studies will be conducted to explore their interaction and identify key molecules.

Keywords: natural killer cell, Schwann cell, pancreatic cancer microenvironment, neural invasion

[PP-062]

Utilization of photoacoustic imaging to evaluate the immunomodulatory effects of Montelukast on T-cell line in the context of Pattern Recognition Receptors

Zulal Celik[1], Dilara Bulut[1], Elif Yaprak Sarac[1], Sude Kara[1], Ozgur Ozdemir[2], Ceren Ciraci[1]
[1]Department of Molecular Biology and Genetics, Istanbul Technical University, Istanbul, Turkey
[2]Department of Electronics and Communication Engineering, Istanbul Technical University, 34469, Istanbul, Turkey

Objectives: Montelukast (MNT), a cysteinyl-leukotriene receptor (Cys-LTR) antagonist, is a well-known allergy drug. It has broader implications for diseases including fibrosis, cardiovascular diseases, cancer, cerebrovascular disease, and immune defenses. However, little is known about its regulatory roles via Pattern Recognition Receptors (PRRs), which needs to be investigated. Photoacoustic imaging (PAI) is a hybrid biomedical imaging technique based on the thermo-acoustic effect induced by laser. It combines the advantages of optical and ultrasonic imaging. Thus, PAI has applications from pre-clinical research to clinical medicine for the detection of cancer, microcirculation abnormalities, and assessment of treatment effectiveness. Our aim was to investigate the effects of MNT on cytosolic PRR, particularly NLR proteins; NLRP3 and NLRC4, both of which are known to form inflammasomes.
Materials & Methods: We conducted molecular assays to determine these effects by qPCR, ELISA, flow cytometry, and immunoblotting. In parallel, the PAI analysis was employed to determine the impact of MNT to show the complementarity and new implication of this emerging tool.
Results: The dose and time effects of MNT on T Jurkat cells were determined by 7-AAD staining using flow cytometry. Treatment of MNT at 10^{-5} M for 48 hours did not change the cell viability. Subsequently, we demonstrated CD25 (activation) and CD40L (late activation) surface expressions to be increased after stimulation. Expression of CYSLTR1 mRNA significantly increased in MNT-treated cells ($p<0.0001$). Interestingly, NLRP3 ($p = 0.0109$) and NLRC4 were elevated at protein level in MNT-treated cells in Jurkat cells. Moreover, a potential signal transduction pathway is currently under investigation.
Conclusions: PAI analysis of MNT-treated cells showed spectral characteristics of photoacoustic signals, which is supporting our molecular findings of MNT in Jurkat cells. Our findings hold great potential for the utilization of PAI technology to analyze different immune cell types, which was rarely used prior to our study.

Keywords: Montelukast, T lymphocytes, photoacoustic imaging, NLRP3, NLRC4

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[PP-063]

Investigation of monocyte amoeboid movement using alginate-PLL hydrogel in lab-on-a-chip

Aslınur Uluçay[1], Devrim Pesen Okvur[2]
[1]Department of Molecular Biology and Genetics, Faculty of Science, Izmir Institute of Technology, Izmir, Turkey
[2]Department of Molecular Biology and Genetics, Faculty of Science, Izmir Institute of Technology, Izmir, Turkey; Initio Cell BV, Leiden, Netherlands

Monocytes are highly mobile cells with amoeboid migration properties, enabling them to navigate through extracellular matrix (ECM) pores and play critical roles in immune responses and inflammation, relevant to both health and disease states. Most existing models utilize hydrogels such as collagen and Matrigel, which are animal-derived, expensive, and susceptible to degradation by enzymes secreted by monocytes. Alginate, a non-biodegradable material, can be modified with positively charged poly-L-lysine (PLL), offering distinct advantages for studying cell motility. This project aims to explore the effects of alginate-PLL complexes on cell migration in three-dimensional (3D) environments using lab-on-chip (LOC) systems, providing a biologically compatible model.
The ICS-Chip device, fabricated from PDMS, features three continuous reservoirs separated by single capillary burst valves and a user-friendly open-top design. This innovative platform facilitates the straightforward placement of hydrogel in the central channel while enabling observation of cell movement dynamics in response to chemoattractants in the side channels. The migration of cells can be tracked, offering valuable insights into cellular behavior under environmental stimuli.
In this study, green fluorescently labeled U937 human monocytes were encapsulated in alginate-PLL hydrogel, and their migration towards serum-free and serum-containing channels was observed. Cells were imaged using confocal microscopy, and data were analyzed with Fiji and R-Studio. Results showed that monocytes exhibited significant migration towards serum-containing media compared to serum-free media. Future studies will expand the application of the ICS-Chip to investigate amoeboid cell migration in various cell types. This platform holds potential for cancer research, immunotherapy, and modeling immune responses, presenting an innovative and sustainable alternative in LOC technology.

Keywords: Alginate, monocyte, amoeboid migration, immunology, microphysiological systems

Design of the Lab-on-Chip (LOC) Device

The figure presents the Invasion/Chemotaxis Single (ICS) Chip, designed to study monocyte migration using an alginate-PLL hydrogel system. The chip has an open-top design with two replicate posts, each containing three interconnected wells. The central channel, where the hydrogel is typically placed, is filled with red dye for visualization.

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[PP-064]

Development of a *Saccharomyces boulardii* probiotic yeast strain expressing interleukin 1 receptor suppressor nanobodies for the treatment of inflammatory bowel diseases

Alp Ertunga Eyupoglu[1], Eymen Gumusdil[1], Mehmet Baha Ozen[2], Batu Erman[2]
[1]Department of Molecular Biology and Genetics, Bogazici University, Istanbul, Turkey
[2]Department of Molecular Biology and Genetics, Acibadem University, Istanbul, Turkey

Saccharomyces boulardii, a non-pathogenic yeast, is a commercially available anti-inflammatory probiotic. S.boulardii has been extensively studied for its ability to support gastrointestinal health and immune regulation. This project aims to develop a novel agent that specifically inhibits the IL-1R using S.boulardii as a host for nanobody expression. Nanobodies are cameloid derived antibodies which have therapeutic applications. Their small size and high affinity confer some advantages over antibodies such as effectively penetrating tumors. Nanobodies targeting the IL-1R pathway involved in inflammatory and autoimmune diseases have been developed. The diseases that are characterized by the dysregulation of the IL-1 pathway, Familial Mediterranean Fever and Behcet's Disease, can be treated by therapeutics targeting this pathway, such as Anakinra and Canakinumab. But these treatment modes have limitations such as reduced efficacy over time and the requirement for frequent administration. Within the project, a cDNA encoding a nanobody that we discovered in our previous studies, which specifically targets the IL-1R and inhibits the IL-1 pathway, will be expressed on the S.Boulardii surface. A new therapeutic strategy will be developed by providing functional nanobody expression in S.Boulardii. Anti-IL-1R nanobody sequences were cloned into the pYDS plasmid compatible with expression in S.cerevisiae. This plasmid encodes a 649 amino acid long stalk region that results in the cell surface expression of these nanobodies. Lyophilized S.boulardii CNCM I-745® was reconstituted in PBS and inoculated in a Sabouraud Dextrose Agar plate and incubated in RT for 48h. Single clones were selected and grown in YPD medium at 37°C. Anti-IL-1R nanobody sequences were cloned into the pYDS plasmid and confirmed by restriction digestion. Selected plasmids were transformed into S.cerevisiae to identify successful surface expression of the nanobody. Further studies will address the functionality of S.cerevisiae compatible plasmids in S.boulardii expression systems and the functional integrity of nanobodies on the yeast surface.

Keywords: Saccharomyces boulardii, IL-1 antagonist, inflammatory bowel diseases

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[PP-065]

Screening and Developing Adhiron Molecules That Bind BTB Protein Binding Domains of ZBTB Transcription Factors

Ilgın Akkaya[1], Görkem Odabaş[1], Batu Erman[2]
[1]Department of Translational Medicine, Graduate School of Health Sciences, Acibadem University, Istanbul, Turkey.
[2]Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Acibadem University, Istanbul, Turkey.

ZBTB proteins are a family of transcription factors that control critical biological processes such as cell differentiation, proliferation, immunoglobulin class switching and the epigenetic regulation of lymphocytes. This highly conserved protein family is associated with multiple malignancies and a rare disease known as Immunodeficiency, Centromeric Instability and Facial Anomalies (ICF) syndrome. This syndrome results from the deficiency of the ZBTB24 protein. Deciphering the functions and molecular interactions of these ZBTB proteins can shed light on developmental processes in the human body and pave the way of novel diagnosis and therapy modalities. We aim to develop affinity reagents, alternative to antibodies. By using molecular engineering and library selection, we develop molecules with high binding specificity that can be used for imaging, detection or molecular inhibition. We created a yeast surface display library of an artificially engineered protein called an adhiron. Adhirons are derived from pytocystatins and their small size, temperature stability and the possibility of bacterial expression make them attractive reagents. We have screened this yeast adhiron display library to find novel binders against the BTB domain of the ZBTB24 protein, using three rounds of magnetic selection followed by three rounds of cell sorting (FACS). We have successfully selected yeast cells expressing ZBTB24 binding adhirons effective at target protein concentrations of 100nM. Sequence analysis of adhiron plasmid DNA from sorted yeast populations revealed that we have identified novel adhirons binding to the ZBTB24 protein. These clones are in the process of being expressed in E.coli and will be characterized in detail. Our future studies include developing these adhirons as detection reagents for the screening of the presence of ZBTB24 protein in ICF Syndrome patients.

This work has been supported by TÜBİTAK Project 123Z458.

Keywords: ZBTB transcription factors, ICF syndrome, Adhiron, Nanobody

[PP-066]

Immunostimulant Potential of Bacterial Extracellular Vesicles Derived From Human Breast Milk Microbiota

Hazal Kibar Dermirhan, Fadime Kıran
Ankara University, Faculty of Science, Department of Biology, Pharmabiotic Technologies Research Laboratory, Ankara, Türkiye

Objective: In recent years, bacterial extracellular vesicles (BEVs) have gained attention as promising natural adjuvant candidates for immunomodulation. This study aims to evaluate the effects of BEVs derived from Pediococcus pentosaceus EIR/HM-1, an isolate from human breast milk microbiota, on RAW 264.7 macrophage cells.

Materials&Methods: BEVs were isolated using ultracentrifugation. The size of the isolated BEVs was analyzed using qNano-exoid, and their protein concentration was determined using a Bradford assay. Atomic Force Microscopy (AFM) was also employed for the characterization of BEVs. Macrophage cells were treated with different concentrations of BEVs, and cytotoxicity was assessed using MTT assay. After 24 hours of stimulation, gene expression levels were determined by qRT-PCR, and cytokine secretions were measured using ELISA. Additionally, nitric oxide (NO) levels were analyzed using a colorimetric assay, while CD80, CD86, and MHC-II markers were tested by flow cytometry.

Results: The protein concentration of BEVs was determined as 1381.628±1.3 µg/mL, and the concentrations between 0.1 and 25 µg/mL displayed no cytotoxicity on macrophage cells. According to the ELISA results, it was shown that P. pentosaceus EIR/HM-1 derived BEVs stimulated the IL-6 and TNF-α production as effectively as LPS. However, the NO assays demonstrated that BEVs were less effective than LPS in inducing NO production. Notably, BEVs upregulated the gene expressions of *TNF-α*, *IL-12*, *NOD2*, *CD206* and *CXCL10* similarly to LPS when compared to the control groups. Additionally, flow cytometry analysis showed that BEVs increased the MHC-II expression in macrophage cells.

Conclusion: This study demonstrates that P. pentosaceus EIR/HM-1-derived BEVs can be utilized as immunostimulant agents for future vaccine applications as well as in other approaches.

Keywords: Bacterial Extracellular Vesicles, Microbiota, RAW 264.7, Immunostimulant

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[PP-067]

The Effects of Exosomes Isolated from Buffy-Coat Concentrates on Neutrophils' Oxidative Burst and ROS Production Capacity

Hilal Vardar[1], İrem Özveri[1], Yağmur Aydın Atalay[1], Yasemin Heper[2], Diğdem Yöyen Ermiş[3], Salih Haldun Bal[4], Haluk Barbaros Oral[3]
[1]Department of Immunology, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey, Experimental Animal Breeding and Research Unit, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey, Experimental and Molecular Immunology Laboratory (Experim), Faculty of Medicine, Bursa Uludag University, Bursa, Turkey, Department of Medicine-Immunology, Institute of Health Science, Bursa Uludag University, Bursa, Turkey
[2]Dr. Rasit Durusoy Blood Bank, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey, Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey
[3]Department of Immunology, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey, Experimental Animal Breeding and Research Unit, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey, Experimental and Molecular Immunology Laboratory (Experim), Faculty of Medicine,
[4]Department of Immunology, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey, Experimental Animal Breeding and Research Unit, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey, Experimental and Molecular Immunology Laboratory (Experim), Faculty of Medicine, Bursa, Turkey, Dr. Rasit Durusoy Blood Bank, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey

Introduction: Buffy-coat concentrates (BFCs) can be transfused to provide neutrophil support in patients with febrile neutropenia when apheresis granulocyte concentrate is not available. In this study, the effects of exosomes in BFCs on the oxidative burst and reactive oxygen species (ROS) production capacity of neutrophils were investigated.
Materials-Methods: Plasma isolated from BFCs (n=80) separated from whole blood collected from healthy volunteers were pooled in equal volumes and exosome isolation was performed. BCA (protein concentration), Western Blot (exosomal proteins such as TSG101, Flotillin-1), Tunable Resistive Pulse Sensing (TRPS) (exosome size and concentration analysis) methods were used to characterize exosomes. The effect of exosomes on the oxidative burst of neutrophils was evaluated by NBT method (n=20); the effect on ROS production was evaluated by using DCFH-DA in flow cytometry (n=1). For both methods, exosomes were cultured with 1x106 neutrophils at 3 different doses (3 µl, 9 µl, 27 µl). For negative control PBS and for positive control PMA was used. For DCFH-DA experiments, neutrophils were separated from peripheral blood with 1.119g/mL ficoll and purified by CD66b+ magnetic activated cell purification (MACS).
Results: Exosomal protein level was measured as 2,500 µg/mL in BCA test. TSG101 and Flotillin-1 were demonstrated by WB. TRPS showed exosome concentration of 3.57E+10 particles/mL and mean size of 128±45.2 nm. Increasing levels of oxidative burst was detected in 3 µL exosome used samples while others showed no detectable changes. However, DCFH-DA result showed that exosomes had lowering effect on ROS production in all titrations without significancy since sample size was low.
This study was supported by Bursa Uludag University Research Projects Coordination Office under the Grant Number TAY-2022-601. The authors thank to BUU BAP Unit for their supports.

Keywords: Buffy-Coat, Neutrophil, Exosome, Oxidative burst

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[PP-068]

The Effects of Erythrocyte Suspensions on THP1 Cell Line via Exosomes

Irem Özveri[1], Hilal Vardar[1], Yasemin Heper[2], Diğdem Yöyen Ermiş[3], Salih Haldun Bal[4], Haluk Barbaros Oral[3]
[1]Department of Immunology, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey, Department of Medicine-Immunology, Institute of Health Science, Bursa Uludag University, Bursa, Turkey Experimental and Molecular Immunology Laboratory (Experim), Faculty of Medicine, Bursa Uludag University, Bursa, Turkey Experimental Animal Breeding and Research Unit, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey,
[2]Dr. Rasit Durusoy Blood Bank, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey, Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey
[3]Department of Immunology, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey, Experimental Animal Breeding and Research Unit, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey, Experimental and Molecular Immunology Laboratory (Experim), Faculty of Medicine, Bursa Uludag University, Bursa, Turkey
[4]Department of Immunology, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey, Experimental Animal Breeding and Research Unit, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey, Experimental and Molecular Immunology Laboratory (Experim), Faculty of Medicine, Bursa Uludag University, Bursa, Turkey, Dr. Rasit Durusoy Blood Bank, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey

Introduction: Transfusion-related immunomodulation (TRIM) is complications of allogeneic blood transfusions (ABT). Decreased function of monocytes and macrophages in the recipient's immune system can be observed after ABT. We investigated the effect of exsosomes isolated from stored erythrocyte suspension (ES) on macrophage polarization.

Materials-Methods: ES was obtained from whole blood obtained from a volunteer donor and divided into two parts. One of these ES was filtered to obtain LR-ESs (leukoreduced). The other ES was not filtered and kept as NL-ESs (non leukoreduced). Exosomes were isolated from NL-ES and LR-ES samples. BCA (protein concentration), Western blot (exosomal proteins such as TSG101, Flotillin-1), Tunable Resistive Pulse Sensing (TRPS) (exosome size and concentration analysis) and flow cytometry methods were used for the characterization of exosomes. 20 µg of exosomes were cultured with 1x106 THP1 cells. CD80, CD86, CD163, CD169, CD206 expressions were analyzed.

Results: Exosomal protein level was measured as 236,45 ug/mL in BCA test. Exosomal proteins CD9 and Flotillin-1 were demonstrated by WB. TRPS showed exosome concentration of 1,02E+10 particles/mL and mean size of exosomes was 109,7 nm and median values was 93 nm. On flow cytometry, CD81 and CD63 expressions levels determined as 96.5 % and 58.4 %, respectively. CD86, CD163, CD169 expressions were significantly decreased with LR-ESs on the THP1. There was no significant difference in CD80 and CD206 expression. While CD169 CD86 expressions decreased, CD163 increased with NL-ES. In line with these results, exosomes isolated from LR-ES and NL-ES has affect on macrophage polarization differently.

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Keywords: Erythrocyte suspension, Exosome, Macrophage, Polarization

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[PP-069]

Characterization of Exosomes Derived from Macrophages Infected with Brucella or Exposed to Inactivated Brucella and Investigation of Their Effect on Macrophage Polarization

Ceren Esen[1], Ceren Esen[2], Ceren Esen[3], Diğdem Yöyen Ermiş[2], Diğdem Yöyen Ermiş[3], Salih Haldun Bal[2], Salih Haldun Bal[3], Ülkü Tüzemen[4], Cüneyt Özakın[4], Haluk Barbaros Oral[2], Haluk Barbaros Oral[3]
[1]Department of Medical-Immunology, Institute of Health Science, Bursa Uludag University, Bursa, Türkiye
[2]Department of Medicine, Laboratory Animal Breeding and Research Unit (DENHAB), Bursa Uludağ University,16059, Görükle, Bursa, Türkiye
[3]Department of Immunology, Faculty of Medicine, Bursa Uludag University, Bursa, Türkiye.
[4]Department of Medical Microbiology Faculty of Medicine, Bursa Uludag University, Bursa, Türkiye.

Introduction: *Brucella* is a type of gram-negative bacteria responsible for the bacterial disease known as brucellosis. The formation of exosomes in cells can alter immune responses during infection. *Brucella* can hide itself in phagocytic cells. In our study, the macrophage polarization of monocyte/macrophage-derived exosomes exposed to *Brucella* bacteria and bacteria-derived outer membrane vesicles (OMVs) was examined.

Method: In our study, THP-1 cells (human monocytic cell line) were cultured separately with inactive *Brucella melitensis* and *Brucella abortus* for 48 hours. Exosomes were isolated from THP-1 cells treated with inactive *Brucella* species using the ultracentrifugation method (100,000xg +4°C). The concentrations of the exosomes were determined using BCA (Bicinchoninic Acid Assay). The isolation of OMVs from live *Brucella* species was conducted using a commercial isolation kit. THP-1 cells were infected with active *Brucella* species. The cells were treated with inactive *Brucella* species, *Brucella*-derived THP-1 exosomes, and bacteria-derived OMVs (each 20ug). After 48 hours of incubation under the conditions, the cells (CD80, CD86, HLA-DR, CD163, CD169, CD206) were analyzed using a flow cytometer.

RESULTS-Conclusion: In conclusion, an increase in CD80-CD86 levels was observed in cells infected with active *Brucella* species, exposed to inactive *Brucella*-derived THP-1 exosomes, and exposed to active *Brucella* OMVs. A statistically significant increase in CD169 levels of the cells was observed, with this increase being less pronounced in THP-1 cells treated with OMV compared to those treated with active *Brucella*species. It has been shown that exosome and OMV structures also exhibit a significant increase in surface markers; however, this increase is less pronounced compared to the increase directly induced by bacteria. ROS-NO levels of cells decreased at the same time.
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Keywords: Infectious diseases, *Brucella*, macrophage, THP-1 cell line

[PP-070]

Unveiling the Molecular Signatures of Extracellular Vesicles in MIS-C Pathogenesis

Tugce Canavar Yildirim[1], Yasemin Ozsurekci[3], Muzaffer Yildirim[1], Volkan Yazar[2], Seza Ozen[3], Kubra Aykac[3], Ali Cengiz[3], Ulku Guler[4], Bekir Salih[4], Mayda Gursel[1], Ihsan Gursel[1]
[1]Basic and Translational Research Program, Izmir Biomedicine and Genome Center, Izmir, Turkey
[2]Department of Molecular Biology and Genetics, Bilkent University, Ankara, Turkey
[3]Division of Pediatric Infectious Diseases, Department of Pediatrics, Faculty of Medicine, Hacettepe University, Ankara, Turkey
[4]Department of Chemistry, Hacettepe University, Ankara, Turkey

MIS-C is distinguished from acute pediatric COVID-19 by its delayed onset and a more pronounced inflammatory response. While acute COVID-19 primarily affects the respiratory system, MIS-C manifests as a multisystemic disorder. Autoantibodies targeting endothelial, immune, and gastrointestinal cells have been implicated in its pathogenesis; however, the underlying mechanisms remain poorly elucidated. We analyzed humoral and plasma cytokine responses in MIS-C patients relative to asymptomatic, mild, and severe pediatric COVID-19 cases. SARS-CoV-2-specific anti-RBD, anti-6p-Spike protein, and anti-nucleocapsid IgM, IgG, and IgA antibody titers of the cohorts (control n=14, COVID-19 n=65, and MISC n=19) were measured by ELISA on admission, and the antibody titers of severe COVID-19 and MIS-C patients were followed longitudinally for 50 days. We compared 18 cytokine levels in the serum of all cohorts using cytometric bead-based multiplex assay panels. For the first time, we performed proteomic profiling of plasma-derived extracellular vesicles (EVs) in MIS-C pediatric patients.
On admission, MIS-C patients exhibited significantly elevated SARS-CoV-2-specific IgG, IgA, and IgM antibody titers compared to COVID-19 patients. Unlike COVID-19, where antibody titers peak at 20–30 days post-infection, MIS-C antibody titers were markedly elevated upon admission and remained sustained, suggesting prolonged exposure to viral antigens. Spike and RBD proteins were detected in EVs from severe COVID-19 and MIS-C patients, indicating persistent viral components in circulation that may contribute to ongoing inflammation. Notably, IL-17A levels were significantly increased in MIS-C patients, implicating this cytokine in disease pathogenesis.
Proteomic analyses revealed that EVs from severe COVID-19 patients activated the NF-κB and IRF pathways, indicative of immune-stimulatory cargo. In contrast, MIS-C-derived EVs selectively trigger IRF signaling without activating NF-κB, revealing distinct molecular profiles. These findings imply that the unique composition of MIS-C EVs underlies dysregulated innate immune responses and hyperinflammation. Further investigation of these EV differences may reveal novel therapeutic targets.

Keywords: COVID-19, MIS-C, Immune dysregulation, Circulating extracellular vesicles

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[PP-071]

Recombinant production of immunogenic MPT64 proteins released during early phase by mycobacteria belonging to *M. tuberculosis* complex as a fusion with self-cleaving intein proteins

Ece Aksoy[1], Burcu Saygıner[1], Tanıl Kocagöz[2], Erkan Mozioğlu[1]
[1]Acibadem Mehmet Ali Aydınlar University, Institute of Health Science, Department of Medical Biotechnology, İstanbul, Türkiye
[2]Acibadem Mehmet Ali Aydınlar University, School of Medicine, Department of Medical Microbiology, İstanbul, Türkiye

M. tuberculosis immunogenic protein (MPT64) plays a crucial role in tuberculosis infections; it is one of the antigens targeted in the tuberculin skin test, also known as purified protein derivative (PPD) skin test. The MPT64 protein is important for both diagnosis and treatment and the novel approach to obtain these proteins with high purity and efficiency continues. In this regard, recombinant DNA technology is widely used, but the use of enzymes such as proteases in the recovery step of affinity-based purification leads to an additional impurity and is limited especially for in vitro applications. In this study, we aimed to produce recombinant MPT64 proteins as a fusion with a tag containing an intein and the chitin binding domain. Intein domains allow the recombinant proteins to self-cleave with the use of DTT without the use of an additional enzyme such as protease, thus making it possible to use it for elution after binding to the chitin column. It is therefore recommended for the production of purer proteins in the literature. This study reports the optimization conditions, production yield and purification of MPT64 expression containing the intein-chitin tag and discusses the challenges encountered, providing useful information for those studying with this immunogenic protein.

Keywords: *Mycobacterium tuberculosis*, MPT64 protein, intein tag, chitin, purification

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[PP-073]

Characterization of Outer Membrane Vesicles (OMVs) from Helicobacter pylori strains isolated from Turkish patients

Pelin Gelmez[1], Zeynep Nur Şentürk[1], Bahar Deniz[1], Mehmet Karaçay[1], Sinem Öktem Okullu[2], Ayça Sayı Yazgan[1]
[1]Department of Molecular Biology and Genetics, Istanbul Technical University, Maslak, Istanbul, Turkiye
[2]Department of Medical Microbiology, Faculty of Medicine, Acibadem Mehmet Ali Aydinlar University, Istanbul, Türkiye

Objective:
Helicobacter pylori is a spiral-shaped, gram-negative bacterium known to cause chronic gastritis and gastric lymphoma. Outer membrane vesicles (OMVs) are small, spherical structures released from the outer membrane of gram-negative bacteria, including H. pylori. Since OMVs mimic the bacterial outer surface, they carry key antigenic components such as virulence factors and nucleic acids. Several clinical strains of H. pylori have been found to contain virulence factors like ureB, cagA, and vacA in their OMVs. Due to their immunogenic properties, OMVs have recently emerged as vaccine candidates against bacterial pathogens. However, there is limited information regarding the characterization of H. pylori and OMVs from Turkish patients. This study aims to characterize OMVs produced by different H. pylori strains isolated from Turkish patients.

Materials/Methods:
H. pylori strains from Turkish patients were cultured in Brucella broth supplemented with FBS and vancomycin. Gram staining was performed for bacterial identification. Bacterial lysates were prepared for protein isolation and analysis. OMVs were isolated using ultracentrifugation. The protein content of OMVs was analyzed using Western blotting for key virulence factors: ureB, cagA, and vacA. OMV size and morphology were characterized via DLS and TEM.

Results:
All strains were gram-negative. CagA, vacA, and ureB were present in both total protein extracts and OMVs of the G27 strain. VacA was detected in two strains and their OMVs, while ureB was found in four strains and the OMVs of two strains. OMV sizes ranged from 20-350 nm.

Conclusion:
OMVs were successfully isolated and characterized. Differences in virulence factor profiles suggest potential variations in immune responses. Future studies will further characterize cagA and vacA in all patient strains and assess immune responses in vivo.

Keywords: Helicobacter pylori, Outer membrane vesicles, Virulence factors

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Keywords: Helicobacter pylori, Outer membrane vesicles, Virulence factors

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[PP-074]

Recombinant production of human VISTA constructs for discovery of new immunotherapeutics

Ali Mert Sencer[1], Sibel Kalyoncu[2]
[1]Izmir Biomedicine and Genome Center; Department of Genome Sciences and Molecular Biotechnology, Izmir International Biomedicine and Genome Institute, Dokuz Eylul University, Izmir, Turkey
[2]Izmir Biomedicine and Genome Center, Izmir, Turkey

VISTA (V-domain Ig suppressor of T cell activation, B7-H5, PD-1H), a B7 family member type I transmembrane protein, plays a crucial role in immune checkpoint functions. Unlike other group members, it is expressed by tumors, T cells and myeloid cells either as a ligand or receptor, or both. These intriguing features make VISTA a promising target in immunotherapy research. To better understand its therapeutic potential, we present a pipeline of design and production of recombinant VISTA constructs.

We aimed to obtain two VISTA constructs, one with His tag, one with IgG1 Fc fusion. We obtained the human VISTA (amino acids 1-184) coding sequence (Uniprot: Q9H7M9) with a C-term linker-Myc-6xHis in pUC57. To facilitate human Fc region cloning, we introduced a XhoI restriction site. This hVISTA-Myc-His construct was then cloned into the pcDNA3.4 mammalian expression vector using restriction insertion cloning at 5'XbaI and 3'AgeI sites. Also, with PCR insertion, we added 5'XhoI and 3'AgeI restriction sites flanking the upper hinge region (EPKSCDKTHT) and the C-term Lys regions of the human IgG1 coding sequences, respectively. This allowed us to delete the C-term Myc-tag-6xHis tail during digestion with XhoI and AgeI restriction enzymes and to ligate the IgG1-Fc coding insert instead.

This hVISTA-Fc-pcDNA3.4 expression vector was transfected into HEK293T cells in suspension culture. The supernatant was harvested after 72 hours of transient expression and subjected to protein-A affinity chromatography purification. The eluted protein solution was then buffer-exchanged into a PBS pH 6.5 solution, a pH that is below the predicted pI value of 7.38. SDS-PAGE gel electrophoresis analysis showed highly pure hVISTA-Fc protein with no observable product loss in purification steps.

We are planning to employ these recombinant hVISTA-Fc and hVISTA-Myc-His constructs in our future immunotherapy research both in the context of cancer and inflammatory disorders.

Keywords: VISTA, immune checkpoint, immunotherapy

[PP-075]

Virulence gene profiling in Helicobacter pylori isolates from Turkish patients: A PCR-based study

Tuana Nur Aydınlar[1], Sümeyye Akçelik Deveci[2], Ayça Sayı Yazgan[2], Sinem Öktem Okullu[3]
[1]Department of Medical Microbiology, Institute of Health, Acıbadem Mehmet Ali Aydınlar University, 34752, Istanbul, Türkiye.
[2]Department of Molecular Biology and Genetics, Istanbul Technical University, Istanbul, Türkiye.
[3]Department of Medical Microbiology, Faculty of Medicine, Acibadem Mehmet Ali Aydinlar University, 34752 Istanbul, Türkiye.

Objectives: Helicobacter pylori is a major human pathogen associated with gastritis, ulcers, and gastric cancer, with distinct virulence genes influencing disease severity and clinical consequences. Adhesin genes such as babA2, babB, babC, hopQ, alpA,alpB, sabA, and sabB help bacteria adhere to the stomach mucosa and are linked to stomach injury. ureA and ureB genes neutralize stomach acid by producing the urease enzyme, while tolB supports cell wall stability, helping the bacteria survive for a long time. Genes such as cagA and oipA increase the risk of stomach cancer by triggering inflammation. In contrast, napA, dupA, and labA genes may increase disease severity by playing a role in immune system evasion mechanisms.
Materials-Methods: This study aimed to detect and analyze the prevalence of virulence-associated genes in five different H. pylori strains that are isolated from Turkish patient cultures. The presence of virulence genes in bacterial strains was analyzed through polymerase chain reaction (PCR) to determine their genetic composition and potential pathogenicity. The strains were cultured in Brucella Broth and pelleted to perform bacterial genomic DNA extraction.
Results and Discussion: The results reveal the distribution of virulence genes in patient isolates, shedding light on H. pylori's genetic diversity and pathogenic potential. All strains carried cagA, napA, OipA, ureA, ureB, tolB, alpA, alpB, sabA, and sabB, while 40% had babA2, babB, and dupA. cagA was detected in 20% of isolates, babC and hopQ in 80%, and labA in 60%. These findings enhance understanding of H. pylori diversity and its role in disease prognosis and treatment.
This study in being supported by The Scientific and Technological Research Council of Türkiye (TUBITAK 1004-KORTUP), Project No:22AG077.

Keywords: Helicobacter pylori, virulence genes, PCR

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[PP-076]

Adjuvant enriched outer membrane vesicles (OMVs) of *Helicobacter pylori* elevated Th1-biased immune response in mice splenocytes

Damla Güvenç[1], Elif Edanur Balçık[1], Sultan Ezgisu Karaman[1], Mehmet Karacay[2], Sinem Öktem Okullu[3], Ayça Sayı Yazgan[2], Banu Bayyurt Kocabaş[1]
[1]Department of Biological Sciences, Host-Microbe Interaction Laboratory, Middle East Technical University, Ankara, Turkey
[2]Department of Molecular Biology and Genetics, Istanbul Technical University, İstanbul, Türkiye
[3]Department of Medical Microbiology, Faculty of Medicine, Acibadem Mehmet Ali Aydinlar University, Istanbul, Türkiye

Helicobacter pylori, a Gram-negative bacterium, is responsible for various gastrointestinal diseases including gastritis, peptic ulcers and even cancer. Its prevalence is estimated to be 80-90% in developing countries and 20-50% in developed countries. Antibiotic combination is an effective treatment though antibiotic resistance is rising. Additionally, there are vaccine candidates to eliminate bacteria and recurrent infections. However, they are ineffective to induce sufficient immune response to elevate a cell (Th1/Th17) mediated and humoral immunity, particularly IgA production. Therefore, we are planning to prepare a vaccine against *H. pylori* using a novel cost-effective platform including outer membrane vesicles (OMVs). They are small spherical particles released from bacteria with content containing various molecules like phospholipids, proteins, and siRNAs. The aim of this project is to develop a safe and effective vaccine prototype against H. pylori infection by combining adjuvants and antigen-carrying *H. pylori* OMVs to achieve antigen-specific immune responses. In this study, we have isolated a specific clinical strain of *H. pylori* OMVs and then treated with 2% sodium deoxycholate for 30 min to dissociate LPS from OMV. Then, splenocytes from C57BL/6 mice were stimulated with OMVs and adjuvants (D and K-type CpG ODN and Alum) at different concentrations for 48h. IFNγ, IL6, TNFα and IFNα/β, levels in culture supernatants of treated splenocytes were checked by ELISA assay and reporter cell line. Combination of OMV with K-type CpG ODN increased IL-6 and IFN-γ secretion indicating Th1-biased immune response activation. Later we will stimulate bone marrow-derived macrophages and dendritic cells (BM-DMs and BM-DCs) to observe APC maturation potential of our adjuvant plus OMV combination. These findings will demonstrate its potential as a vaccine to elicit a balanced and effective immune response against *H. pylori*.

Keywords: *Heliobacter pylori*, OMV, Th-1 response

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[PP-077]

Discovery of Peptide-based Inhibitor Targeting the Human IL18:IL18Rα Complex

Zeynep Şevval Düvenci[1], Ahmet Can Timuçin[2], Emel Timuçin[1]
[1]Department of Biostatistics and Bioinformatics, Institute of Health Sciences, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkey
[2]Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkey

Uncontrolled release of IL18, proinflammatory cytokine that belongs to the IL1 family and plays crucial role in inflammation, is associated with severe inflammatory diseases and therefore therapeutic approaches that block IL18 activity are important for treatment of these diseases. To target the IL18:receptor complex to modulate IL18 activity, binding proteins of human and/or viral origin that bind to IL18 have emerged. In the study of proof of that concept, the efficacy and safety of biological molecules targeting IL18 have been demonstrated in a variety of diseases with high IL18 activity, and directly targeting IL18 has proven to be a valid strategy in clinical models. Despite these positive developments, studies on stopping IL18 activity with different and smaller molecules compared to biologics are still increasing. In that context, two small molecules that block a region on surface of IL18 were found in a recent study. However, to effectively inhibit the IL18:receptor protein-protein binding surface, which has a very large area, compounds that exceed typical drug sizes are needed. Peptide-based compounds can be tested as IL18 inhibitors due to their small intermolecular size with biologics. Peptides are particularly advantageous compared to small molecules, because they can more effectively cover the protein-protein interaction surface than do small molecules and they have relatively low production costs and can more easily penetrate into tissues than do biologics. However, peptides targeting the IL18:receptor complex have not been previously studied. At this point, the aim of this study was to discover peptide-based inhibitors targeting IL18 and its receptor complexes and to evaluate their in vitro efficacy.

Keywords: interleukin-18, inflammation, peptide, protein-protein interactions, structure-based drug design

[PP-078]

Nanoparticle-based vaccine development with liposome-loaded CpG ODN and Poly(I:C) against Rotavirus for neonates

Havva Homak, Damla Güvenç, Elif Edanur Balçık, Sultan Ezgisu Karaman, Banu Bayyurt Kocabaş
Department of Biological Sciences, Host-Microbe Interaction Laboratory, Middle East Technical University, Ankara, Turkey

Rotavirus is a non-enveloped double-stranded RNA virus spreading through fecal-oral route and causing diarrhea-related death in children under 5 years old worldwide. Concerns about the safety of live attenuated vaccines with side effects and inadequate protection against different strains in developing countries result in a low vaccination rate, even though it can achieve immunity against viruses early in life. Therefore, we aim to develop a novel nanoparticle-based subunit vaccines containing local antigen with multiple pattern recognition receptor (PRR) agonists as nucleic acid based adjuvants loaded into liposomes. In our previous findings, we determined combination of PRR agonists, poly(I:C) (TLR3) and CpG ODN (TLR9), elevated levels of IL-6, IL-12 and type I and II interferons (IFN-α/β and IFN-γ), as well as upregulated co-stimulatory molecules (CD80 and CD86) on splenocytes of BALB/c neonates (5-7 days old) and adult (6-8 weeks old) mice. In our in vivo studies, poly(I:C) and/or CpG ODN with model antigen OVA either free or co-encapsulated into neutral liposomes were injected i.p. twice at two-week intervals into neonate mice. Two weeks after booster vaccination, experiment was ended by collecting spleen, mesenteric lymph nodes and blood sera from vaccinated mice. Splenocytes and lymphocytes were re-stimulated with OVA to determine Ag-specific IFN-γ and IL4 production via ELISA. OVA-specific Ig response was detected from sera by Ig ELISA. We found that poly(I:C) and CpG ODN co-encapsulated into liposomes increased IgG2a/IgG1 ratio by upregulating Th1-immunity related cytokine production. Next, we will administer parental vaccination to investigate its impact on neonates. Additionally, subsequent studies will involve testing the rotavirus antigen VP6 to show specific efficacy against rotavirus infection.

Keywords: Rotavirus, Parental vaccination, Nanoparticle-based vaccine development, Liposomes

[PP-079]

Optimization studies for recombinant production of ESAT-6 protein in *Escherichia coli*, which plays an important role in *tuberculosis* immunopathology

Lara Güneş, Burcu Saygıner, Ceren Kocabaş, Erkan Mozioğlu
Medical Biotechnology Department, Institute of Health Sciences, Acibadem University

Early Secreted Antigenic Target 6 (ESAT-6) is a 6-kDa protein which is secreted by *Mycobacterium tuberculosis* as part of the ESX-1 secretion system. Due to its ability to influence host immunological responses, enhance bacterial survival, and facilitate spread within the host, it is important in understanding the virulence and pathogenesis of *tuberculosis*. In diagnostic tests for tuberculosis, ESAT-6 is commonly used as it has significant antigenic properties. Since it is important to obtain high quantities of these immunologically important proteins, optimization studies on the production of ESAT-6 proteins recombinantly in *Escherichia coli (E.coli)* were carried out in this study. Although *E. coli* is widely used as microbial factories for the production of recombinant proteins, there are limitations such as inclusion body production in the production of foreign proteins such as those belonging to *M. tuberculosis*. In this study, optimization studies on the production of ESAT-6 proteins in *E.coli* with high efficiency were performed and the proteins could be produced in soluble form.

Keywords: Immunogenic recombinant ESAT-6 protein production, optimization, *Escherichia coli*

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[PP-080]

M1/M2 macrophage polarization in early *Candida* immune responses

Florabelle Ruano Cabarrubias[1], Anna Luelly Baumer Machado[1], Attila Gácser[2]
[1]Department of Biotechnology and Microbiology, University of Szeged, Szeged, Hungary; HCEMM-USZ, Fungal Pathogens Research Group, Szeged, Hungary
[2]Department of Biotechnology and Microbiology, University of Szeged, Szeged, Hungary; HCEMM-USZ, Fungal Pathogens Research Group, Szeged, Hungary; HUN-REN-SZTE Pathomechanisms of Fungal Infections Research Group, University of Szeged, Szeged, Hungary; Competence Centre for Molecular Biology, Bionics, and Biotechnology, University of Szeged, Szeged, Hungary

Macrophages can polarize into either classically activated (M1) or alternatively activated (M2) phenotypes in response to microenvironmental signals. *Candida* species, including *Candida albicans* and *Candida parapsilosis*, are fungal pathogens responsible for the majority of systemic yeast infections in nosocomial settings. During invasion, these pathogens interact with monocytes, which can influence macrophage polarization. In this study, we aimed to evaluate macrophage polarization during the early stages of *Candida*-macrophage interactions.

To investigate this, peripheral blood mononuclear cell-derived monocytes (PBMC-DMs) and M1-like macrophages were co-incubated with *C. albicans* (SC5314) and *C. parapsilosis* (CLIB 214). Macrophages were immunolabeled with CD68 (macrophage marker), CD86 (M1 marker), and CD163 (M2 marker) and analyzed using flow cytometry. Additionally, cytokine levels of TNF-α and IL-6 (pro-inflammatory) as well as TGF-β and IL-10 (anti-inflammatory) were measured.

Our results revealed that the ratio of M1 and M2 populations was not significantly altered by exposure to *C. albicans,* regardless of whether PBMC-DMs or M1-like macrophages were used. The M1/M2 ratios remained similar to those observed in untreated controls. In contrast, when PBMC-DMs were co-incubated with *C. parapsilosis*, a significant increase in the M2-type population was observed, suggesting a tolerogenic effect. Interestingly, M1-like macrophages co-incubated with *C. parapsilosis* exhibited a mixed M1+/M2+ population, indicating a transitional state.

Cytokine analysis further highlighted these differences. PBMC-DMs exposed to *C. parapsilosis* showed elevated levels of IL-6 and IL-10, while M1-like macrophages displayed increased levels of TNF-α, IL-6, and IL-10. These findings suggest overlapping M1/M2 functions and indicate that *C. parapsilosis* promotes an M2 phenotype in PBMC-DMs while inducing a transitional M1/M2 state in M1-like macrophages.

Our study demonstrates that *Candida* species elicit distinct macrophage polarization responses during early fungal-macrophage interactions. These differences reflect the unique pathogenic strategies employed by *Candida* species and provide insights for developing targeted therapeutic strategies to combat *Candida* infections.

Keywords: Monocytes, Macrophage, Polarization, Candida, Pro-inflammatory, Anti-inflammatory

[PP-081]

Optimization studies of recombinant production of CFP-10, a T cell stimulatory protein for interferon gamma (IFN-γ) production in tuberculosis infections, in *Escherichia coli*

Muhammed Yusuf Yılmaz, Burcu Saygıner, Erkan Mozioğlu
Medical Biotechnology Department, Institute of Health Sciences, Acibadem University, İstanbul, Türkiye

Tuberculosis is one of the deadliest infectious diseases. Early treatment is crucial and rapid diagnosis plays a key role in enabling timely intervention. For this purpose, the detection of biomolecules specific to *Mycobacterium tuberculosis* causing tuberculosis is the main method for rapid diagnosis. 10-kDa culture filtrate protein (CFP-10) is one of these biomolecules since CFP-10 forms a 1:1 heterodimeric complex with early secreted antigenic target 6 kDa (ESAT-6) and this complex plays a critical role in the virulence of the *M. tuberculosis* infection. It is therefore necessary to produce this immunogenic protein to be used as target molecules for both diagnosis and treatment. For this purpose, in this study, the recombinant production of CFP-10 protein in *Escherichia coli* was optimized to use this protein in further studies. Firstly, the CFP-10 gene was cloned into the pET His6 TEV LIC cloning vector by performing LIC cloning. After confirmation by Sanger Sequencing, plasmid vectors were transferred into the *E.coli* Bl21 bacterial cells for expression. The optimum expression condition for the recombinant production of CFP-10 protein was investigated by using different culture media, different concentrations of isopropyl β-D-1-thiogalactopyranoside (IPTG), at different incubation temperatures/times. As a result, the optimization studies required to obtain the target CFP-10 protein in *E.coli* in high yield have been completed, and our findings are valuable for both clinical and industrial applications related with *M. tuberculosis*.

Acknowledgments
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Keywords: Immunogenic recombinant CFP-10 protein production, *Mycobacterium tuberculosis, Escherichia coli*

[PP-083]

α-Tocopherol-13'-Carboxychromanol Modulates Inflammatory Response of Macrophage in Acute Lung Injury

Ferah Kaplanseren[1], Ibrahim Isot[1], Bengu Cetinkaya[1], Anil Can[2], Devrim Oz Arslan[3], Merve Acikel Elmas[2], Erdi Sozen[4]
[1]Department of Biochemistry, Faculty of Medicine, Marmara University, Istanbul, Türkiye
[2]Department of Histology and Embryology, School of Medicine, Acıbadem Mehmet Ali Aydinlar University, Istanbul, Türkiye
[3]Department of Biophysics, School of Medicine, Acıbadem Mehmet Ali Aydinlar University, Istanbul, Türkiye
[4]Genetic and Metabolic Diseases Research Center (GEMHAM), Marmara University, 34854, Istanbul, TURKEY

Acute lung injury (ALI) is a severe inflammatory condition characterized by increased pro-inflammatory cytokine levels, immune cell infiltration, and tissue damage. α-Tocopherol-13'-carboxychromanol (αT-13'-COOH), a long-chain metabolite of α-tocopherol, has been shown to exhibit anti-inflammatory properties, but its role in macrophages and ALI remains unexplored. This study aims to evaluate the therapeutic potential of αT-13'-COOH in vitro and in vivo experimental models and to elucidate its effects on macrophage-related inflammation response.
In this direction, in vitro experiments were performed to investigate the immunomodulatory effects of α-13'-COOH on bone-marrow derived macrophages. Additionally, ALI was induced in mice via lipopolysaccharide (LPS) administration, followed by αT-13'-COOH treatment. Histopathological analysis of lung tissue was performed to assess structural damage while mRNA levels of inflammatory cytokines were determined by qRT-PCR.
Our preliminary findings suggest that α-13'-COOH treatment significantly reduces lung inflammation, modulates macrophage activity, and promotes tissue repair. By providing novel insights into the role of α-13'-COOH in ALI, this study offers potential therapeutic implications for inflammatory lung diseases and lays the foundation for further investigations into its immunoregulatory mechanisms.

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Keywords: α-Tocopherol-13'-carboxychromanol, Inflammation, Acute Lung Injury (ALI), Macrophage modulation, Cytokines

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[PP-084]

Clinical and Cytokine Profiles of Pediatric Tularemia in Türkiye

Muzaffer Yildirim[1], Ozlem Cakici[4], Kubra Aykac[2], Bera Enes Seyrek[3], Ismail Cem Yilmaz[1], Tugce Canavar[1], Irem Evcili[1], Gokcenaz Tuluoglu[1], Osman Oguz Demir[5], Emine Hafize Erdeniz[6], Yasemin Ozsurekci[2], Ihsan Gursel[1]
[1]Izmir Biomedicine and Genome Center, DEU Saglik Kampusu, Balcova, Izmir, Turkiye
[2]Department of Pediatric Infectious Diseases, Hacettepe University Faculty of Medicine, Ankara, Türkiye
[3]Hacettepe University School of Medicine, Ankara, Türkiye
[4]Department of Pediatric Infectious Diseases, Sivas Numune Hospital, Sivas, Türkiye
[5]Department of Pediatric Diseases, Afyon University Faculty of Medicine, Afyon, Türkiye
[6]Department of Pediatric Infectious Diseases, Ondokuz Mayıs University Faculty of Medicine, Samsun, Türkiye

Tularemia, caused by Francisella tularensis, has reemerged among pediatric patients in Türkiye between 2023 and 2024, with some cases demonstrating significant resistance to treatment. This study aimed to evaluate clinical characteristics, cytokine profiles, and treatment responses in 40 pediatric patients, categorized as good (n=26) or poor (n=14) responders. The predominant clinical presentation was oropharyngeal tularemia, particularly prevalent in the Central Anatolia region, with seasonal peaks between January and April. Natural stream water exposure and living in rural areas were identified as common risk factors. Laboratory findings indicated that patients with good responses had higher median CRP levels (10 mg/dL) compared to poor responders (6.1 mg/dL), while white blood cell counts were similar. Elevated serum levels of IFN-γ and IL-6 were observed at hospital admission compared to healthy controls, reflecting robust innate and adaptive immune activation. Following treatment, significant decreases were noted in IL-6, IL-9, IL-10, and IFN-γ levels, indicating resolution of inflammation and immune regulation. Treatment predominantly involved ciprofloxacin and gentamycin, with a median duration of 14 days. Surgical drainage was significantly more common in the poor response group, suggesting that suppurative lymphadenopathy predicts suboptimal antibiotic response. The study highlights that successful antimicrobial therapy mitigates inflammatory cytokine responses, restoring immune homeostasis. No deaths occurred in either group, underscoring the effectiveness of appropriate antibiotic treatment. These findings emphasize the importance of early diagnosis, tailored therapeutic strategies, and public health interventions to mitigate risk factors, particularly in endemic regions. Further research is warranted to investigate age-specific

Keywords: Pediatric tularemia, Francisella tularensis, cytokines, IFN-γ, IL-6, clinical outcomes

[PP-085]

Understanding the roles of Eosinophil-like cells in inflammatory disease models through NOD2/CARD9 axis

Edanur Barutçu, Elif Yaprak Sarac, Sude Kara, Ceren Ciraci Mugan
Department of Molecular Biology and Genetics, Istanbul Technical University,Istanbul, Türkiye

Objectives: Numerous studies on the pathogenesis of inflammatory diseases have focused on the adaptive immune responses, however innate immunity has recently been in the limelight. Also, viral infections may influence treatment options for these diseases. While eosinophils are primarily associated with parasitic infections, emerging data suggest contributions to antiviral immunity. Our study aimed to analyze the reciprocal relationships of NOD2 and CARD9, known as susceptibility genes in inflammatory diseases, in the context of viral immunity.

Materials-Methods: NOD2 and CARD9 in EoL-1 cell line were downregulated using increasing doses (10-20-50 nM) of NOD2 and CARD9 siRNA. To assess optimal knockdown efficiency, mRNA levels were analyzed by real-time quantitative PCR and accompanied by semi quantitative indirect immunocytochemistry. Following siRNA transfection, EoL-1 cells were stimulated with viral ligands R848 (TLR7/8 ligand) and ssRNA40 (TLR8 ligand). Cytokine levels in response to viral stimulation were measured by ELISA.

Results: NOD2 and CARD9 were significantly downregulated using 20 nM siRNA ($p<0.05$). siRNA mediated single downregulation of NOD2 or CARD9 suggested a mutual regulation of one another ($p<0.05$). IL-1β and IFNα cytokines had decreasing trend, although non-significant ($p>0.05$).

Conclusion: Our findings suggested that NOD2 and CARD9 may play interconnected roles in eosinophil-like cells, influencing inflammatory responses to viral stimuli, which may highlights their potential as therapeutic targets in inflammatory and viral-mediated diseases.

Keywords: NOD2, CARD9, Eosinophils, inflammatory diseases

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[PP-086]

Transcriptomic analysis of the effect of TRAIL on human macrophages and its potential impact on lymphocyte activation and recruitment

Taliha Inan[1], Sinem Gunalp[1], Ahmet Bursali[2], Gokhan Karakulah[1], Duygu Sag[3]
[1]Department of Genomic Sciences and Molecular Biotechnology, Izmir International Biomedicine and Genome Institute, Dokuz Eylul University, Izmir, Turkey; Izmir Biomedicine and Genome Center, Izmir, Turkey
[2]Izmir Biomedicine and Genome Center, Izmir, Turkey
[3]Department of Genomic Sciences and Molecular Biotechnology, Izmir International Biomedicine and Genome Institute, Dokuz Eylul University, Izmir, Turkey; Izmir Biomedicine and Genome Center, Izmir, Turkey; Department of Medical Biology, Faculty of Medicine, Dokuz Eylul University, Izmir, Turkey

Objective:
TNF-related apoptosis-inducing ligand (TRAIL) can trigger apoptosis or induce survival by binding to DR4/DR5 death receptors. Our group has previously shown that TRAIL promotes the polarization of human macrophages towards the pro-inflammatory M1 phenotype by upregulating M1 markers, while downregulating M2 markers. However, the impact of this effect on the function of macrophages such as lymphocyte activation and recruitment remain unclear. This study aims to analyze the effect of TRAIL on human macrophages in recruiting and activating T and NK cells at the transcriptomic level.

Materials-Methods:
Human blood monocyte-derived macrophages were stimulated with 200 ng/ml soluble TRAIL for 8 hours and the changes in the molecules associated with T and NK cell activation/ recruitment were analyzed by RNA-seq and qPCR.

Findings and Results:
Of all macrophage markers obtained from the RNA-seq data, the expression rates of genes relevant to T and NK cell activation/ recruitment that reached significant expression levels were examined. Our data showed that TRAIL stimulation increased the expression of RSAD2, CCL4, CCL3, IL-18, and ICAM-1 in primary human macrophages. qPCR analyses confirmed that TRAIL stimulation upregulated the expression of CCL4 and CCL3 in human macrophages. Surprisingly, RSAD2 expression was downregulated, while IL-18 and ICAM-1 exhibited no statistically significant changes by qPCR.

Conclusion:
Our study demonstrates that TRAIL stimulation leads to increased gene expression of the chemokines CCL4 and CCL3 in primary human macrophages, suggesting enhanced lymphocyte recruitment function. Our findings suggest that TRAIL influences macrophage-driven immune responses, offering insights into its immunomodulatory potential.

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Keywords: TRAIL, Macrophage, Lymphocyte, Chemokine

[PP-087]

Exploring the Role of RIG-I in Human Microglial Response Under Hypoxic Conditions

Yagmur Ozcelebi, Esra Kantar, Edanur Barutcu, Ceren Ciraci
Istanbul Technical University, Department of Molecular Biology and Genetics, Istanbul, Turkiye

Objectives: Pattern recognition receptors (PRRs), a critical component of innate immunity, have the ability to recognize pathogen-associated molecular patterns (PAMPs) to generate inflammatory responses. Retinoic acid inducible gene I (RIG-I) is a PRR that induces an immune response against RNA viruses, resulting in tissue damage. Hypoxia - lack of oxygene - is another mechanism that leads to tissue damage. This study unveils the roles of RIG-I in the hypoxia induced inflammation, autophagy and Akt/mTOR signaling.
Materials-Methods: Human microglial clone 3, HMC3, cell line was adapted as a model. Both upregulation by Poly (I:C) transfection; and downregulation by siRNAs, targeting RIG-I were performed in HMC3 cells before chemical hypoxia induction.
Results: Hypoxia induction was verified by Hif-1α expression. Data regarding downregulation of RIG-I by siRNAs suggested that RIG-I inhibited phosphorylated Akt and mTOR proteins and induced autophagy. Moreover, Hif-1α expression decreased by siRNA-mediated knockdown. Also, upregulation of RIG-I by Poly (I:C) transfection altered expression of phosphorylated Akt and mTOR proteins, further presenting the regulation of Akt/mTOR pathway by RIG-I as well as inflammatory responses.
Conclusion: RIG-I may have regulatory roles in autophagy and inflammation under hypoxia.

Keywords: RIG-I, Hypoxia, Autophagy, Microglia

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[PP-088]

How prosaptide affects the phenotype and maturation of bone marrow-derived dendritic cells

Berra Nur Ateşok[1], Elif Nedret Keskinöz[2], Devrim Öz Arslan[3]
[1]Department of Medical Biotechnology, Institute of Health Sciences, Acibadem Mehmet Ali Aydinlar University, Istanbul, TURKEY
[2]Department of Anatomy, School of Medicine, Acibadem Mehmet Ali Aydinlar University, Istanbul, TURKEY
[3]Department of Biophysics, School of Medicine, Acibadem Mehmet Ali Aydinlar University, Istanbul, TURKEY

Objective: Prosaposin, a lysosomal glycoprotein, functions both as a precursor of sphingolipid activator proteins, known as saposins, and as a secreted signaling molecule. Extracellular prosaposin promotes cell survival, lipid metabolism, immune modulation, and neuroprotection. Recent studies have shown that prosaposin and prosaposin-derived 14-mer peptide (TX14), regulate macrophage activation, cytokine secretion, and inflammatory responses in macrophages, while their impact on dendritic cells (DCs) remains elusive. This study investigates the effect of prosaptide TX14(A) on the phenotype and maturation of bone marrow-derived dendritic cells (BMDCs).

Materials-Methods: BMDCs were isolated from BALB/c mouse femurs and tibias and then cultured with GM-CSF to generate immature DCs (iDCs). On day 7, iDCs were treated with 1 µg/ml LPS for 48 hours to obtain mature DCs (mDCs). Morphological changes were examined via light microscopy, and cell viability was evaluated by MTT assay to determine the optimal concentration of prosaptide TX14(A). Phenotyping of iDCs and mDCs was performed by flow cytometry using CD11c, MHC II, CD40, CD80, and CD86 antibodies.

Results: iDCs and mDCs were successfully generated after 7 and 9 days of culture, respectively, exhibiting differential expression of CD40, CD80, CD86, and MHC II. Subsequently, the cells were exposed to prosaptide TX14(A) (ranging from 10 to 5,000 nM) for 24 and 48 hours. However, the MTT assay did not demonstrate any cytotoxic effects within this concentration range. Notably, prosaptide modulated DC surface marker expression levels at 50 and 500 nM concentrations of prosaptide TX14(A) at 24 and 48 hours. Furthermore, its potential mechanisms of action are currently being investigated.

Conclusions: This study highlights for the first time the role of prosaptide in modulating DC activation and phenotype, offering new insights into its immunomodulatory properties and therapeutic applications.

Keywords: Prosaposin, Prosaptide, Dendritic Cells, Immune Modulation, Maturation

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[PP-089]

The differential virulence of *Fusarium* strains causing corneal infections and plant diseases is associated with accessory chromosome composition

Dilay Hazal Ayhan[1], Serena Abbondante[2], Domingo Martínez Soto[3], Siyuan Wu[4], Ricardo Rodriguez Vargas[3], Shira Milo[4], Katherine Rickelton[4], Michaela Ellen Marshall[2], Marina Campos Rocha[5], Neta Shlezinger[5], Eric Pearlman[2], Li Jun Ma[4]
[1]Biochemistry and Molecular Biology, University of Massachusetts Amherst, Amherst, MA, USA; Molecular and Cellular Biology, University of Massachusetts Amherst, Amherst, MA, USA; Graduate School of Natural and Applied Sciences, Acibadem University, Istanbul, Türkiye
[2]Physiology and Biophysics and Ophthalmology, University of California, Irvine, USA
[3]Department of Microbiology, Centro de Investigación Científica y Educación Superior de Ensenada (CICESE), Ensenada, Baja California, Mexico
[4]Biochemistry and Molecular Biology, University of Massachusetts Amherst, Amherst, MA, USA; Molecular and Cellular Biology, University of Massachusetts Amherst, Amherst, MA, USA
[5]The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Jerusalem, Israel

Fusarium oxysporum is a cross-kingdom pathogen. While some strains cause disseminated fusariosis and blinding corneal infections in humans, others are responsible for devastating vascular wilt diseases in plants. To better understand the distinct adaptations of *F. oxysporum* to animal or plant hosts, we conducted a comparative phenotypic and genetic analysis of two strains: MRL8996 (isolated from a keratitis patient) and Fol4287 (isolated from a wilted tomato [*Solanum lycopersicum*]). *In vivo* infection of mouse corneas and tomato plants revealed that, while both strains cause symptoms in both hosts, MRL8996 caused more severe corneal ulceration and perforation in mice, whereas Fol4287 induced more pronounced wilting symptoms in tomato. *In vitro* assays using abiotic stress treatments revealed that the human pathogen MRL8996 was better adapted to elevated temperatures, whereas the plant pathogen Fol4287 was more tolerant to osmotic and cell wall stresses. Both strains displayed broad resistance to antifungal treatment, with MRL8996 exhibiting the paradoxical effect of increased tolerance to higher concentrations of the antifungal caspofungin. We identified a set of accessory chromosomes (ACs) and protein-encoding genes with distinct transposon profiles and functions, respectively, between MRL8996 and Fol4287. Interestingly, ACs from both genomes also encode proteins with shared functions, such as chromatin remodeling and post-translational protein modifications. Our phenotypic assays and comparative genomics analyses lay the foundation for future studies correlating genotype with phenotype and for developing targeted antifungals for agricultural and clinical uses.

Keywords: fungal pathogens, cornea, eye, host-pathogen interaction, genomics, myeloid cells

[PP-090]

Immunotoxic effects of Ochratoxin A on natural killer cell activity

Kardelen Uzunkaya[1], Betül Çakıcı[2], Elif Çelik[2], Tolga Sütlü[2], Ibrahim Yaman[1]
[1]Boğaziçi University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics, İstanbul, Türkiye
[2]Acıbadem Mehmet Ali Aydınlar University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics, İstanbul, Türkiye

Objective: Ochratoxin A (OTA), a prevalent mycotoxin produced by Aspergillus and Penicillium fungi, exhibits intricate immunotoxicity. Its toxicity encompass reduced immune cell viability and proliferation, increased inflammatory responses, and diminished cytotoxic activity. Despite extensive research on OTA-induced immunotoxicity in primary NK cells of rodents, the underlying molecular mechanisms remain enigmatic. The primary objective of our study is to elucidate OTA-mediated innate immunotoxicity at a molecular level by investigating its impact on NK cell activation.
Materials-Methods: The sublethal OTA doses for NK-92 cells were determined through cell viability assays employing propidium iodide staining. Subsequently, the alterations in the functional activity of OTA-treated NK-92 cells, specifically in terms of granzyme B expression and degranulation, were investigated. This was achieved by analyzing the intracellular granzyme B levels and surface CD107a expression, respectively. The altered NK-92 cell phenotype was examined by surface staining of specific activating receptors in response to varying doses of OTA treatments. The differentially expressed proteins involved in the PI3K/Akt, MAPK/ERK, and NF- κB signaling pathways in OTA-treated NK-92 cells were investigated by western blot analysis, both in the presence and absence of NK cell stimulation.
Results: Our findings revealed a dose-dependent reduction in the survival of OTA-treated NK-92 cells. OTA treatment induced a pronounced increase in degranulation, granzyme B expression, and surface levels of the NKp46 receptor in NK-92 cells. Notably, OTA exposure led to significant alterations in protein levels of the PI3K/Akt, MAPK/ERK, and NF-κB pathways in NK-92 cells.
Conclusion: Our findings demonstrate that OTA exposure of NK-92 cells leads to a reduction in cell viability, accompanied by differential protein expression and elevated degranulation, granzyme B, and NKp46 levels. These insights provide crucial molecular mechanisms underlying the innate immunotoxicity of OTA on NK cell activity, potentially elucidating its immunotoxic effects and their potential association with carcinogenic risks.

Keywords: Ochratoxin A (OTA), natural killer (NK) cells, immunotoxicity

[PP-091]

Exopolysaccharides From *Limosilactobacillus reuteri* EIR/Spx-2 as a novel immunostimulatory agent

Hazal Kibar Dermirhan, Fadime Kıran
Ankara University, Faculty of Science, Department of Biology, Pharmabiotic Technologies Research Laboratory, Ankara, Türkiye

Objective: Recent studies highlight the immunomodulatory potential of exopolysaccharides (EPS) derived from probiotic bacteria as natural, biocompatible, and effective candidates. This study evaluates the in vitro immunostimulant effects of EPS derived from *Limosilactobacillus reuteri* EIR/Spx-2 on RAW 264.7 macrophage cells.

Materials&Methods: EPS was extracted from the supernatant of *L. reuteri* EIR/SPX-2, purified using ethanol precipitation, and dialyzed. The total carbohydrate content of EPS was measured using the phenol-sulfuric acid method. Extracted EPS was characterized by FTIR, HPLC, and thermogravimetric analysis. The cytotoxicity profile was assessed in RAW 264.7 macrophage cells using the MTT assay. Following 24 hours of stimulation, gene expression levels were determined by qRT-PCR, and cytokine secretion was measured using ELISA. Additionally, nitric oxide (NO) levels were determined using a colorimetric assay, while CD80, CD86, and MHC-II markers were analyzed by flow cytometry.

Results: The total carbohydrate content of EPS was found to be 12034.04±1.9 mg/L. Concentrations between 1 and 100 µg/mL displayed no cytotoxicity on macrophage cells. ELISA results showed that EPS (10-100 µg/mL) enhanced the IL-6 and TNF-α secretion, suggesting an immune response similar to that of LPS. NO assays revealed that 100 µg/mL EPS significantly increased NO production. EPS also upregulated the gene expressions of *TNF-α*, *IL-10*, *iNOS*, *TLR4*, *ARG1*, *CD206* and *CXCL10*, similar to LPS, when compared to the control groups. Additionally, flow cytometry analysis showed that EPS increased MHC-II and CD80 expression in macrophage cells to a greater extent than LPS, while CD86 expression was found similar to that of LPS.

Conclusion: This study suggests that *L. reuteri* EIR/Spx-2-derived EPS could serve as an immunostimulant agent to enhance vaccine immunogenicity in the future. Further research is needed to elucidate its mechanisms and expand its applications.

Keywords: Bacterial exopolysaccharides, RAW264.7, Microbiota, Immunostimulant

[PP-092]

Investigation of Antioxidant and Immunomodulatory Potential of Microbiota-Derived Postbiotics

Buse Safrancı, Gülsün Bolat, Hazal Kibar Demirhan, Fadime Kıran
Ankara University, Faculty of Science, Department of Biology, Pharmabiotic Technologies Research Laboratory, Ankara, Türkiye

Objective: The present study aims to evaluate the antioxidant and immunomodulatory potential of postbiotics derived from *Lactiplantibacillus plantarum* EIR/HM-1 strain, an isolate from the fecal microbiota of breast-fed infant, on hydrogen peroxide-stimulated DLD-1 (ATTC CCL-221) cell line and LPS-induced RAW264.7 mouse macrophage cells.

Material&Methods: Postbiotics were extracted from the supernatant of *L. plantarum* EIR/HM-1 by centrifugation, and lyophilized. DLD-1 and RAW264.7 cells were treated with varying doses of postbiotics, and their cyotoxicity was evaluated by MTT assay. After 24 hours of stimulation, the antioxidant effect of postbiotics on hydrogen peroxide-stimulated DLD-1 cells and the immune-stimulatory roles on LPS-induced RAW264.7 were evaluated by qRT-PCR analyses.

Results: According to the results, postbiotics increased the expression of *CAT* and *SOD1* genes in DLD-1 cells against oxidative damage caused by hydrogen peroxide. Additionally, the expression of *NRF2* and *KEAP* genes, which directly affect ROS homeostasis by regulating antioxidant defense systems through various mechanisms, was also increased. Furthermore, it was determined that postbiotics promoted the proliferation of RAW 264.7 mouse macrophage cells and exhibited an immune-stimulating effect by promoting the production of key cytokines involved in the host defense response, such as *IL-12*, *IL-6*, *IL-1β*, and *TNF-α*.

Conclusion: This study suggests that *L. plantarum* HM-1-derived postbiotics may reduce oxidative stress and modulate immune responses. The findings indicate their potential for prophylactic or therapeutic applications in inflammatory bowel diseases (IBD). Further research is needed to clarify their mechanisms and explore their clinical relevance in IBD treatment.

Keywords: Microbiota, Postbiotic, Immunmodulatory, Antioxidant, DLD-1, RAW264.7

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[PP-093]

Anti-inflammatory effects of postbiotics derived from *Limosilactobacillus vaginalis* isolated from the oral microbiota of healthy individuals against periodontal disease

Emine Omeroglou[1], Hatice Büşra Esen[2], Fadime Kıran[1]
[1]Gaduate School of Natural and Applied Sciences, Department of Biology, Pharmabiotic Technologies Research Laboratory, Ankara University, Ankara, 06110, Turkey
[2]Institute of Health Sciences, Department of Stem Cell and Regenerative Medicine, Ankara University, 06100, Ankara, Turkey.

Objective: Periodontal diseases are multifactorial conditions linked to bacterial biofilms and the host's inflammatory response, leading to gingival inflammation and alveolar bone loss. Lipopolysaccharides (LPS) from bacteria like *Porphyromonas gingivalis* activate immune cells, triggering pro-inflammatory cytokine release and tissue destruction. Recently, postbiotics, metabolites from probiotics, have emerged as potential biotherapeutic agents for oral inflammation. This study evaluates the anti-inflammatory effects of postbiotics from *Limosilactobacillus vaginalis* on human periodontal ligament stem cells (hPDLSCs).
Materials and methods: *Limosilactobacillus vaginalis* was isolated from the oral microbiota of healthy individuals and identified through 16S rRNA gene sequencing. Postbiotics were obtained by centrifugation and lyophilization. For *in vitro* studies, hPDLSCs were isolated from periodental ligament tissues of third molar roots from orthodontic patients and characterized using flow cytometry. The effect of postbiotics (10-5000 µg/mL) on hPDLSCs viability was assessed using the MTT assay. hPDLSCs were treated LPS derived from *P. gingivalis* LPS (1 µg/mL) with and without postbiotics, and the levels of cytokines were determined by RT-qPCR and ELISA.
Results : Flow cytometry analysis for hPDLSC characterization confirmed that cells exhibited high expression of mesenchymal markers CD73 (80.50%), CD90 (86.90%), and CD105 (94.81%), with minimal expression of hematopoietic (CD34: 2.20%, CD45: 0.79%) and endothelial (CD31: 0.65%) markers. Postbiotic mediators had no significant effect on hPDLSC viability up to 1000 µg/mL. RT-qPCR analysis revealed that postbiotics downregulated the expressions of *IL-6* and *TNF-α* genes while upregulating the *IL-10* levels. ELISA analysis was also confirmed that postbiotics increased the anti-inflamtory cytokine secretion while decreased the pro-inflammatory cytokines.
Conclusion: Postbiotics derived from the oral microbiota may exhibit therapeutic potential in periodontal inflammation. These findings suggest that postbiotics could be used as adjunctive agents in the treatment of periodontal diseases. However, further studies are required to fully understand the mechanism of action.

Keywords: Inflammation, Oral microbiota, Periodontal disease, Postbiotics

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[PP-094]

Oxidative phosphorylation affects *Helicobacter*-activated B cell survival and differentiation

Zeynep Nur Senturk, Bahar Deniz, Eliz Onbas, Minel Horozlar, Ayça Sayi Yazgan
Department of Molecular Biology and Genetics, Istanbul Technical University, Istanbul, Turkey

Objective: Oxidative phosphorylation plays a critical role in immune cell survival, differentiation, and function. Studies indicate that inhibition of electron transport system (ETS) Complex I with rotenone reduces IL-10 production in CpG (TLR9 ligand) stimulated B cells. In addition, rotenone-treated B cells modulate IFN-γ production in $CD4^+$T cells within a co-culture system. However, the effect of rotenone on Helicobacter-activated B cells is unclear. Therefore our study aimed to explore the impact of rotenone on the survival, proliferation, and IL-10 secretion of *Helicobacter felis* (*H. felis*) stimulated B cells.
Materials-Methods: B cells were magnetically sorted from the spleen of VertX IL10 ^{egfp} mice and stimulated with *H. felis* antigen (10 ug/ml), TLR2-ligand PAM3CSK4 (2.5 ug/ml) or TLR4-ligand LPS (5 ug/ml) in the presence or absence of different doses of rotenone for 24&48 hours. For the proliferation assay, cells were labeled with CFSE and cultured for 72 hours. Viability and IL-10 production were assessed using 7-AAD staining and IL-10 GFP signal detection via flow cytometry.
Results: Our data show that rotenone significantly decreases B cell viability and IL-10 production, especially at the 48-hour time point. The viability is reduced from 80-85% to 50-70% with 80 nM rotenone and to 30-50% with 160 nM rotenone. Additionally, increased rotenone concentrations reduced B cell proliferation from 60% to 20–40%. While IL-10 production in PAM3CSK4 and *H. felis*-activated B cells was markedly reduced, LPS-activated B cells showed no change.
Conclusion: Inhibition of mitochondrial Complex I impaired B cell survival, IL-10 production, and proliferation. These findings suggest that mitochondrial metabolism is essential for the expansion and IL-10 production of *Helicobacter*-activated B cells. Further studies are required to elucidate the impact of rotenone-treated, *Helicobacter*-stimulated B cells on $CD4^+$T cell responses.
Acknowledgments: This study was supported by the TUBITAK. (Project Number: 119S447)

Keywords: B cell, *Helicobacter*, Mitochondria, Rotenone

[PP-095]

Development of affinity-tagged VHH proteins for capture of GFP-tagged proteins

Eymen Gümüşdil[1], Alp Ertunga Eyüpoğlu[1], Batu Erman[2]
[1]Molecular Biology and Genetics, Bogazici University, Istanbul, Turkey
[2]Department of Molecular Biology And Genetics, Acıbadem Mehmet Ali Aydınlar University, Istanbul, Turkey

Antibodies play a role in regulating immune responses in the body. Due to their selective binding properties to other molecules, they are widely used in various scientific methods such as immunostaining and blotting. Traditional antibodies consist of light and heavy chain pairs with variable and constant regions and are approximately 150 kDa in size. However, animals like camelids also have antibodies about 70 kDa in size, made solely of heavy chains. Proteins called nanobodies have been developed by utilizing the variable regions of these heavy chains. Nanobodies, exhibit superior tissue penetration due to their small size (approximately 15 kDa), and disulfide bonds in their structure provide stability and durability. These features have significantly increased the use of nanobodies in scientific research. Studies conducted in our laboratory showed that some members of the ZBTB protein family form condensed structures in cells due to phase separation mediated by their BTB domains. These ZBTB proteins are also tagged with GFP. Based on this, the individual and condensed structures of these proteins will be used to produce/test nanobodies that recognize the GFP tag. To produce nanobodies that are suitable for co-immunoprecipitation (Co-IP), commercially available GFP-binding nanobody will be cloned into the Pet22b plasmid with two separate tags, Strep-Tag II and Avi-Tag. Inside of the cell the fusion protein will bind to GFP-tagged ZBTB proteins through the nanobody, while the Strep-Tag II and Avi-Tag enable the nanobody to bind to streptavidin beads. This construct will be prepared for use in Co-IP methods. The choice of different tags for streptavidin binding is due to the weaker binding of Strep-Tag II (~100 μM) compared to the strong biotin-streptavidin interaction provided by Avi-Tag (approximately 10 fM through biotinylation). At the end of this project, the purification capacity of the nanobodies will be evaluated via mass spectrometry (MS).

Keywords: Immunoprecipitation, Antibodies, Nanobodies, Mass Spectrometry, Affinity Labels

[PP-096]

Gestational Diabetes Mellitus Alters Mitochondrial Function in in Cord Blood Mononuclear Cells

Musa Çelik[1], Berkcan Koç[1], Saygın Abalı[2], Bengisu Guner Yilmaz[3], Serdar Beken[4], Devrim Oz Arslan[5]
[1]Department of Biophysics, Institute of Health Sciences, Acıbadem University, Istanbul, Turkey
[2]Department of Pediatrics, School of Medicine, Pediatric Endocrinology, Acıbadem University, Istanbul, Turkey.
[3]Department of Pediatrics, School of Medicine, Acıbadem University, Istanbul, Turkey.
[4]Department of Pediatrics, School of Medicine, Neonatology, Acıbadem University, Istanbul, Turkey.
[5]Department of Biophysics, School of Medicine, Acıbadem University, Istanbul, Turkey.

Introduction: Gestational diabetes mellitus (GDM) is a common endocrine disorder during pregnancy that can lead to various metabolic consequences for the fetus and during the postnatal period. Cord blood mononuclear cells (CB-MNCs), which play a crucial role in neonatal immune responses, are particularly susceptible to metabolic alterations. This study aims to investigate the effects of GDM on mitochondrial function in CB-MNCs, which may contribute to immune dysfunction in neonates.

Methods: CB-MNCs were isolated from fetuses of healthy and GDM mothers. Mitochondrial superoxide production (MitoSOX), mitochondrial mass (MM), and mitochondrial membrane potential (MMP) were quantitatively assessed using flow cytometry with mitochondria-specific fluorescent probes. To further evaluate mitochondrial function, MMP and MitoSOX levels were measured following exposure to electron transport chain (ETC) complex inhibitors, including rotenone, oligomycin, and antimycin.

Results: We observed reduced MMP and MitoSOX levels in the GDM group, indicating impaired mitochondrial activity. Exposure to mitochondrial inhibitors indicated significant alterations in ETC function. In the presence of rotenone, both MMP and MitoSOX levels were lower in the GDM group, while antimycin treatment led to a higher increase in MitoSOX levels, pointing to heightened oxidative stress. FCCP-induced MMP reduction was more pronounced in the GDM group, and oligomycin treatment resulted in a greater MMP increase compared to controls. These findings suggest mitochondrial dysfunction in CB-MNCs of GDM-exposed neonates, which may compromise immune cell metabolism and function.

Conclusion: GDM-induced metabolic disturbances impair mitochondrial function in CB-MNCs, potentially disrupting immune programming and leading to long-term immunometabolic consequences. Further research is needed to explore the link between maternal metabolic disorders and neonatal immune development.

Keywords: Gestational Diabetes Mellitus, Mitochondrial Function, Cord Blood Mononuclear Cells, Electron Transport Chain

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[PP-097]

A network-based gene set activity scoring method for single-cell RNA sequencing datasets

Zeynep Garan[1], Ebru Kocakaya[1], Şadi Güngör[2], Yasin Kaymaz[2]
[1]Graduate School of Natural and Applied Sciences, Department of Biotechnology, Ege University, İzmir Turkey
[2]Faculty of Engineering, Bioengineering Department, Ege University, Izmir Turkey

Single-cell RNA sequencing (scRNA-seq) enables detailed examination of the functions and interactions of cells within complex tissues by measuring the entire transcriptomic gene expression of individual cells. Tracing transcripts back to their source cells allows researchers to identify unique gene expression profiles in highly heterogeneous samples. It plays a critical role in uncovering rare cell types and dynamic cell states. Many studies use scRNA-seq data to explore cell heterogeneity, discover subpopulations, and compare gene set activities to gain insights into diseases, biological processes, or cellular mechanisms. However, they often focus solely on comparing gene expression across different conditions by examining gene sets with high activation under specific conditions while others overlook the interaction networks between genes. These shortcomings hinder a complete understanding of diseases and biological processes at the single-cell level and prevent the in-depth study of functional subgroups of cells.
The proposed approach presents a new single-cell gene set activity scoring method for scRNA-seq data. This method integrates gene expression values by weighting the relationships between genes within gene sets using a network-based algorithm. Our method allows genes interacting with many others to be considered more important given their expression levels in a cell. In addition, this approach smoothens the effect of sparsity in the data by computing a normalized gene set activity score and allows for a holistic observation of biological processes by evaluating both the expression states of genes and their relationships.
In this study, we explored neutrophils in a large lung cancer atlas and their tumor microenvironment depended states with the gene set activity perspective. Our initial analysis validated our approach to be potentially useful for revealing new biomarkers of neutrophils associated with their immunosuppressive functions. These findings emphasize the importance of cellular heterogeneity and gene-gene interactions in understanding biological processes.

Keywords: Single-cell RNA sequencing (scRNA-seq), Pathway scoring, Cellular heterogeneity, Gene-gene interactions, Bioinformatics, Network-based analysis

[PP-098]

Investigating the Mechanism of OTA-Induced eIF2α Phosphorylation and Global Translation Inhibition

Semanur Karayılan, Ibrahim Yaman
Department of Molecular Biology and Genetics, Bogazici University, Istanbul, Turkey

Ocratoxin A (OTA) is a mycotoxin produced by some Penicillium and Aspergillus genera of fungi, known for its genotoxic, hepatotoxic, immunotoxic, nephrotoxic, and carcinogenic effects. OTA induces eIF2α phosphorylation, a key event for inhibiting 5'cap-dependent translation initiation. It can also cause oxidative stress, ER stress, and possible empty tRNA accumulation through Phe-tRNA synthetase inhibition, which may activate eIF2α kinases. While OTA is known to inhibit protein synthesis, its effects on global translation rate and eIF2α phosphorylation are not fully explored. Preliminary studies in our lab show a time-dependent effect on eIF2α phosphorylation and global translation rates. This project aims to uncover the activated eIF2α kinases and mechanisms involved.
Specific inhibitors A92, C16, and GSK2606414 were used to investigate the roles of GCN2, PKR, and PERK respectively in OTA-induced eIF2α phosphorylation and global translation inhibition. These inhibitors were applied to human kidney (HK-2) and rat liver progenitor (WB-F344) cell lines in a dose-dependent manner, 2.5 hours before OTA treatment. Changes in eIF2α, ERK, and Akt phosphorylation levels were assessed via Western Blot analysis, and translation rates were measured using the SUnSET (SUrface SEnsing of Translation) method with anti-puromycin antibody.
The results suggest that GCN2 and PKR kinases are involved in OTA-induced eIF2α phosphorylation and translation inhibition at certain doses, although inhibiting neither completely abolished the effects. Inhibition of both kinases decreased Akt and mTOR activation, indicating possible cross-signaling, while PERK inhibition showed no notable effects.
Our findings are shedding light on OTA toxicity mechanisms in HK-2 and WB-F344 cell lines, specifically how it affects global translation through eIF2α phosphorylation. The significant roles of GCN2 and PKR suggest potential redundancy among eIF2α kinases. Time-dependent experiments with the most effective doses will be conducted for further insights. Moreover, each kinase will be knocked down using shRNA to further elucidate their individual contributions.

Keywords: Ochratoxin A (OTA), eIF2α, Global Translation

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[PP-099]

Analysis of the Interaction Between PTP-PEST and PSTPIP1 in an *In Vitro* Cell Culture Model

Zeynep Tonbul, Elif Güzel, Eda Tahir Turanlı, Umut Inci Onat
Institute of Natural and Applied Science, Acıbadem Mehmet Ali Aydınlar University, Istanbul, Turkey;Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics, Acıbadem Mehmet Ali Aydınlar University, Istanbul, Turkey

Objective

PSTPIP1 (Proline-Serine-Threonine Phosphatase Interacting Protein 1) encodes a protein that plays a crucial role in the immune system and cellular signaling. We previously showed that PSTPIP1 p.Arg228Cys variant has an increased interaction with pyrin in FMF patients. The aim of this project is to investigate the relationship between the rare missense variants p.Arg228Cys (NM_003978.5: c.C682T, rs781341816) and p.Ala372Val (NM_003978.5: c.C1115T, rs200188483) in PSTPIP1 and PTP-PEST in vitro cell culture models.

Materials and Methods

Vectors containing wild type and variant forms of PSTPIP1 were transfected into HEK293FT cell line using lipofectamine. Co-immunoprecipitation was utilized using PSTPIP1 (SantaCruz, B-10) antibody. The protein-antibody mixture was then incubated with Dynabeads® Protein G, and immunoblotting was performed using PSTPIP1 and PTP-PEST antibodies. GAPDH was used as the loading control.

Results

Overexpression of PSTPIP1 was determined by comparing the transcript levels between the transfected and untransfected samples (2-ΔΔCT method). According to the immunoblotting results, no significant difference was observed between the wild-type and variant forms in their interaction with PTP-PEST. The results were analyzed using GraphPad Prism 8 software (GraphPad Software, San Diego, USA)

Conclusion

In this study, we investigated the interaction of wild-type and PSTPIP1 variants with PTP-PEST in HEK293FT cells. These findings suggest that the variants do not alter the interaction of PSTPIP1 with PTP-PEST under the experimental conditions used. Further studies, including functional assays and structural analyses, may be required to elucidate the potential impact of these variants on PSTPIP1-associated signaling pathways.

Keywords: PSTPIP1, PTP-PEST, Co-IP

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[PP-100]

Impact of *LAMA5* Variants on Immune Cell Infiltration using 2D Blood Brain Barrier Culture Model of Multiple Sclerosis

Umut Voyvoda[1], Sevim Naz Karisik[1], Meziyet Dilara Reda[1], Hanbike Efe[1], Kerem Cotuk[2], Betul Cakici[1], Ahmet Can Timucin[4], Ugur Uygunoglu[3], Melih Tutuncu[3], Aksel Siva[3], Umut Inci Onat[4], Eda Tahir Turanli[5]
[1]Department of Molecular Biology and Genetics, Graduate School of Natural and Applied Science, Acibadem University, Istanbul, Türkiye
[2]Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Acibadem University, Istanbul, Türkiye
[3]Department of Neurology, Istanbul University Cerrahpaşa School of Medicine, Istanbul, Türkiye
[4]Department of Molecular Biology and Genetics, Graduate School of Natural and Applied Science, Acibadem University, Istanbul, Türkiye; Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Acibadem University, Istanbul, Türkiye
[5]Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Acibadem University, Istanbul, Türkiye; Institute of Natural and Applied Science, Acibadem University, Istanbul, Türkiye

Objective: MS pathogenesis involves the compromise of the blood-brain barrier (BBB) integrity, allowing peripheral immune cells to breach the barrier and infiltrate the central nervous system, thereby initiating neuroinflammation and neurodegeneration in the brain and spinal cord. The primary objective of the study is to examine how variants of the LAMA5 gene, identified in MS families, influence immune cell infiltration across the BBB.

Materials-Methods: The LAMA5 p.Thr3126Ile missense mutation, present in MS families, was introduced into a human umbilical vein endothelial cell (HUVEC) line using cytosine base editing. Wild-type and mutant HUVEC cells were then co-cultured with peripheral blood mononuclear cells (PBMCs) in a Transwell co-culture system, and PBMC migration through the Transwell was optimized by stimulating the cells with TNFα and IFNγ. Additionally, the expression of *ICAM1*, *VCAM-1*, *CLDN5*, *ITGB1*, and *ITGAL* genes, known to be involved in lymphocyte migration across the BBB through interaction with LAMA5, was evaluated in both wild-type and mutant HUVEC cells using quantitative PCR.

Results: The results revealed that PBMC migration from the modified HUVEC cells was 25% higher compared to the wild-type co-culture conditions. Furthermore, in comparison to the wild-type, the expression of *ICAM-1*, *VCAM-1*, and *CLDN5* was significantly reduced in the modified HUVEC cells, while the expression of *ITGB1* and *ITGAL* was significantly increased.

Conclusion: It has been observed that *LAMA5* variant increase lymphocyte infiltration in the BBB 2D co-culture system.. To further explore immune cell infiltration, PBMCs will be analyzed by flow cytometry to identify distinct subtypes. Moreover, gene expression levels will be examined at the protein level through Western blot analysis. These forthcoming studies will provide valuable insights into the contribution of *LAMA5* variants to the pathogenesis of MS.

Keywords: Multiple Sclerosis, Blood Brain Barrier, Cell Migration, Neuroinflammation, Base Editing

[PP-101]

Identification of the mechanisms controlling the polymerization of ZBTB transcription factors

Egemen Erim[1], Ege Ezen[2], Tuğçem Müge Gür[1], Batu Erman[3]
[1]Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Boğaziçi University, İstanbul, Turkey
[2]Center of Experimental Rheumatology, Department of Rheumatology, University Hospital Zurich, University of Zurich, Switzerland
[3]Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Acıbadem Mehmet Ali Aydınlar University, İstanbul, Turkey

The human genome encodes 49 different ZBTB family proteins which function in cell proliferation, DNA damage responses, and gene repression. Their N-termini contain BTB domains that are responsible for protein-protein interactions and their C-termini contain zinc finger motifs that bind nucleic acids. These proteins form obligate homodimers but previous work from our lab has shown that some family members such as PATZ1 (ZBTB19) and PATZ2 (ZBTB24) can also form heterodimers. To expand the list of heterodimer forming ZBTB proteins, we tested all combinations of family members using the AlphaFold 2 algorithm and identified ZBTB9 as a promiscuous family member predicted to heterodimerize with other ZBTB proteins. Curiously, the BTB domain of ZBTB9, when transfected into HEK293 or HeLa cells formed large condensates observed under the confocal microscope. We identified a motif in helix A4 of ZBTB9, containing a Ser residue responsible for condensate formation. This motif is conserved 19 ZBTB family members that forms a polymerization interface in structural predictions. To identify if condensates formed by ZBTB9 and other motif containing family members contains multiple proteins, we performed co-transfection experiments with the ZBTB9 BTB domain with other ZBTB family members that either contain or do not contain this conserved motif. When BTB domains that contain motifs (ZBTB5, ZBTB6 and ZBTB8A) were co-expressed with ZBTB9, we did not observe condensate co-localization. On the other hand, ZBTB9 BTB condensates recruited BTB domains lacking the conserved motif (BCL6, PATZ1, PATZ2, PLZF). Interestingly, when ZBTB9 BTB domains lacking an NLS are co-expressed, they can recruit BTB domains containing the polymerization motif as well as those that do not contain the motif in cytoplasmic condensates. To assess the potential function of these condensates we are determining their role in DNA damage responses.
This project was funded by TÜBİTAK grant 123Z458.

Keywords: ZBTB9, heterodimer, polymerization, condensate

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[PP-102]

Evaluation of HLA-B27 results using flow cytometry and sequence specific primer-PCR methods in a university hospital

Maşide Tokgöz[1], Burcu Kaya[1], Server Yağcı[1], Halil Özbaş[2]
[1]Clinical Microbiology, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey
[2]Medical Genetics, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

Objective:

Seronegative spondyloarthropathies (SSA) are a group of autoimmune inflammatory diseases in which rheumatoid factor or antinuclear antibodies are not detected. Common features of SSAs include familial predisposition and HLA-B27 positivity HLA-B27 can be detected by various methods with similar sensitivity and specificity with genotyping methods. However, these methods are expensive and time-consuming compared to flow cytometry. HLA-B27 can be detected in peripheral blood lymphocytes using HLA-B27 monoclonal antibody in flow cytometry.

The aim of this study is to compare the HLA-B27 results obtained by flow cytometry and HLA-B27 genetic analysis and to examine the characteristics of patients.

Materials-Methods:

HLA-B27 results obtained by FACS Canto flow cytometer (Becton Dickinson, USA) and by Real-Time PCR method between January 2020 and December 2024 were examined retrospectively. The demographic and clinical characteristics of the patients were registered.

Results:

Data of 32 patients were examined. Both tests were reported as positive in 4/32 patients (12.5%) and negative in 28/32 patients (87.5%). 18 of 32 patients were female (56.25%) and 14 were male (43.75%). The median age for all patients was found to be 41.5. It was seen that 17 (53.12%) of the tests were requested from the rheumatology clinic. 8 (25%) of the patients had at least one diagnosis related to rheumatological disease (ankylosing spondylitis, arthritis, erythema nodosum, etc.). The mean age of patients with positive results was found to be 36.25.

Conclusions:

Flow cytometry is an auxiliary test that can provide rapid and reliable results in the diagnosis of HLA-B27-related diseases when compared to genetic analyses. Comprehensive studies with larger patient populations are needed to get more extensive data.

Keywords: flow cytometry, HLA-B27, seronegative spondyloarthropathies

Figure 1. Distribution of gender by age in the patient population

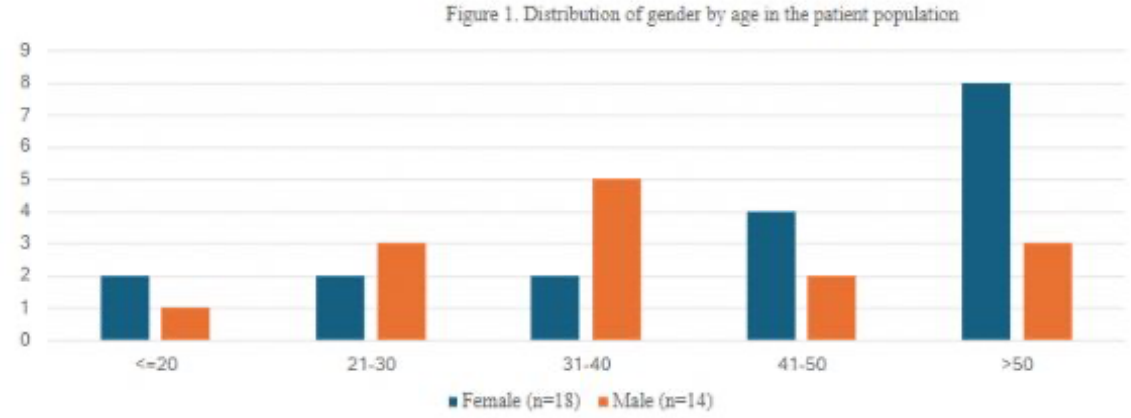

Table 1. Distribution of HLA-B27 results by gender in the patient population

HLA-B27 result/Gender	Female	Male	Total
Positive	2	2	4
Negative	16	12	28
Total	18	14	32

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[PP-103]

Assessment and Clinical Monitoring of a Case with a Homozygous Piezo1 Gene Mutation

Anıl Işık[1], Alp Kazancıoğlu[2], Canan Caka[2], Ceren Üstün[2], Gulnar Aliyeva[2], Elif Soyak Aytekin[2], Sevil Oskay Halaçlı[1], Saliha Esenboğa[2], Deniz Cagdas[2]
[1]Child Health Institute, Department of Pediatric Basic Sciences, Hacettepe University, Ankara, Turkey
[2]Child Health Institute, Department of Pediatric Immunology, Hacettepe University, Ankara, Turkey

Piezo proteins, characterized as mechanosensitive cation channel polypeptides, function as permeable channels for +1 and +2 cations. Piezo1 mutations have been linked to hereditary xerocytosis, congenital lymphatic dysplasia, iron metabolism disorders, and malaria resistance.

A 5-year-old boy with consanguineous parents, a sister with hemolytic anemia, and an intracranial cyst detected on prenatal ultrasound was evaluated for neonatal respiratory distress. He was diagnosed with profound anemia (Hb: 6.6 g/dL) and received an erythrocyte suspension. His peripheral blood smear showed mild polychromasia, anisocytosis, teardrop cells, and macrocytosis. During follow-up, he developed meningitis, suspected to be hospital-acquired. Both direct and indirect Coombs tests were negative, and pyruvate kinase and G6PD enzyme levels were normal.

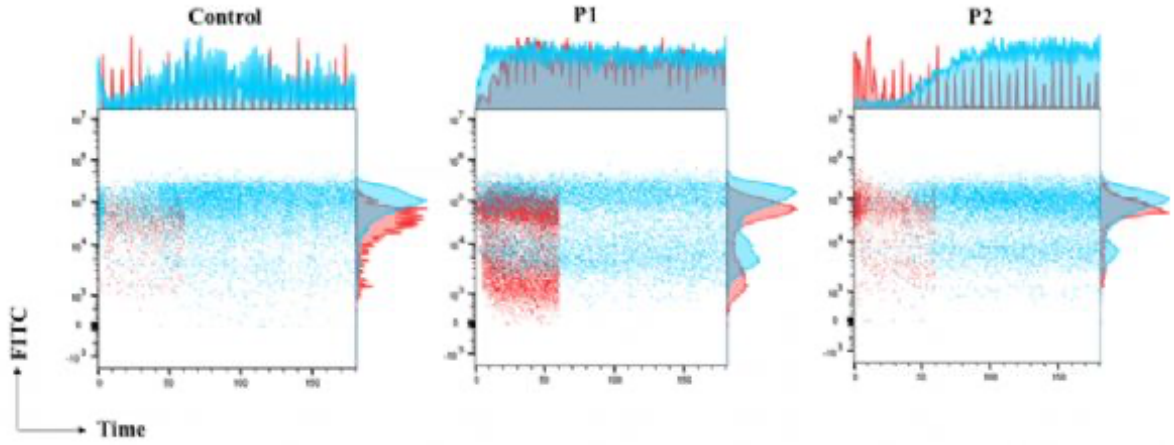
Diagnosed with chronic nonspherocytic hemolytic anemia and congenital dyserythropoiesis, he did not require further transfusions. Due to recurrent tonsillitis and otitis, he was evaluated by immunology, and trimethoprim-sulfamethoxazole prophylaxis was initiated. Neutropenia and hypogammaglobulinemia were detected. After adenoidectomy and tympanostomy tube placement, he was screened for periodic fever disorders. Despite recurrent fevers, throat cultures showed no bacterial growth. MEFV gene analysis revealed no mutations, and follow-up for PFAPA and autoinflammatory diseases is ongoing.

The patient was also diagnosed with malnutrition, motor developmental delay, and secundum atrial septal defect (ASD). At 4.5 years, he was hospitalized with pneumonia and has since been receiving IVIG therapy (400 mg/kg monthly).

Genetic testing identified a homozygous c.427G>A (p.Ser1425Asn) mutation in Piezo1. This gene, associated with bone marrow failure syndromes, is believed to contribute to the patient's anemia, neutropenia, and hypogammaglobulinemia.

Keywords: PIEZO1, Calcium metabolism, Neutropenia, Primary immunodeficiency

CaFlux analysis



PBMC Ca Flux findings prior to and following stimulation.

[PP-105]

Functional compansation of immune pathways in MyD88-Deficiency

Irem Evcili[1], Ersin Gul[2], Buse Duru Aras[1], Yildiz Camcioglu[3], Ihsan Gursel[1], Ayca Kiykim[3], Bilgi Gungor[1], Mayda Gursel[1]
[1]Izmir Biomedicine and Genome Center, Izmir, Turkey
[2]Department of Biological Sciences, Middle East Technical University, Ankara, Turkey
[3]Department of Pediatrics, Infectious Diseases, Clinical Immunology and Allergy Division, Cerrahpasa Medical School, Istanbul University, Istanbul, Turkey

MyD88 (myeloid differentiation primary response protein 88) is an essential adaptor protein involved in signaling of most Toll-like receptors (TLRs) and IL-1 receptor family. Humans with inborn MyD88 deficiency suffer from life-threatening infections caused by a small subset of pathogens. However, these patients may exhibit immunity against viral, fungal and parasitic pathogens, suggesting presence of compensatory MyD88-independent immune pathways. In this study, we investigate the cellular response of a MyD88 patient *in vitro* to gain a better understanding of underlaying molecular mechanisms. We compare data from the initial study with a follow up study conducted 10 years later. We isolated peripheral blood mononuclear cells (PBMCs) from healthy controls and a MyD88-deficient patient, stimulating them with diverse ligands for 24 hours. Cytokine concentrations in the supernatant were quantified using Cytometric Bead Array (CBA) and ELISA. We demonstrate that MyD88-independent nucleic acid sensing pathways are functional, suggesting that these intracellular sensors may contribute to resistance to viral infections in the MyD88-deficient patient. Furthermore, the non-canonical, AIM-2 and NLRC4 inflammasomes can be directly activated in patient cells, implying uncoupling from TLR signaling pathways, which may account for the observed discordance in infection susceptibilities of MyD88-deficient mice and humans.

Keywords: MyD88, Toll like receptors, Primary Immunodeficiency

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[PP-106]

A very rare form of T cell lymphopenia: *RHOH* deficiency

Canberk Ipşir, Ismail Yaz, Dilan Inan, Sevil Oskay Halaçlı, Barış Ulum, Elif Soyak Aytekin, Saliha Esenboğa, Deniz Çağdaş
Division of Immunology, Department of Pediatric Basic Sciences, Institute of Child Health, Hacettepe University, Ankara, Turkey.

Epidermodysplasia verruciformis (EV) is a rare skin disease characterized by decreased ability to defend against and eradicate particular human papillomavirus types, as well as persistent infection and risk of cutaneous dysplasia or neoplasia. Typical EV (biallelic loss-of-function mutations in Transmembrane channel-like protein 6(TMC6/EVER1) or Transmembrane channel-like protein 8(TMC8/EVER2) and Calcium- and integrin-binding protein 1(CIB1)) and atypical EV(defects in *STK4, RHOH, CORO1A, ITK, TPP2, DCLRE1C, LCK, RASGRP1, DOCK8* genes) may develop due to given gene defects. Here, we present two RHOH patients with recurrent infectious diseases, including pneumonia, persistent EV, and T cell deficiency.

Case 1

A 32-year-old male patient, who has parental consanguinity, admitted to the outpatient clinic with the complaints of recurrent upper/lower respiratory tract infections(RTI) since the age of 4. He had a history of lobectomy at the age of 15 and several cryotherapy sessions since the age of 19 due to multiple warts on his hands and feet. Physical examination showed secretory rales in the lower zones of the right lung and bilateral diffuse rhonchi. In the immunological evaluation, atypical form of T cell deficiency was considered. Homozygous missense *RHOH* gene defect (c.451T<C) was detected. T cell proliferation was low compared to control following stimulation with anti-CD3 and anti-CD28.

Case 2

A 10-year-old girl, born to consanguineous parents, was hospitalized due to CMV pneumonia when she was 3 months old and admitted to the outpatient clinic with recurrent lower RTI. Whole exome sequencing (WES) showed a homozygous missense c.510C>G (A170A) variant in the *RHOH* gene.

Result

The Ras homologous family member H (RhoH) is an adapter protein mainly expressed in hematopoietic cells. It is responsible for recruiting ZAP70 to the T cell receptor complex leading to a defect in TCR signaling. We here showed two novel RHOH defects in two siblings diagnosed with T cell deficiency.

Keywords: Inborn Errors of Immunity, RHOH, ZAP70, Combined Immunodeficiency, Epidermodysplasia verruciformis

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