

5th INTERNATIONAL MOLECULAR IMMUNOLOGY & IMMUNOGENETICS CONGRESS (MIMIC-V)

Abstract Book

20-22
October
2022

Sabancı Culture Palace
Dokuz Eylul University
İZMİR / TURKIYE

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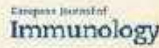
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5th INTERNATIONAL MOLECULAR IMMUNOLOGY & IMMUNOGENETICS CONGRESS (MIMIC-V)

20-22 October, 2022
Sabancı Culture Palace, Izmir, Türkiye

SCIENTIFIC PROGRAMME

THURSDAY, OCTOBER 20th, 2022

08:30 - 11:00	REGISTRATION	
11:00 - 12:00	OPENING CEREMONY	
	OPENING SPEECHES	Chairs: BARBAROS ORAL, İHSAN GURSEL
	OPENING CONFERENCE	Chair: GUNNUR DENİZ
12:00 - 13:00	T CELL DIFFERENTIATION AND IMMUNE REGULATION	FEDERICA SALLUSTO (EFIS PRESIDENT, SUJ) (ONLINE)
13:00 - 13:30	LUNCH BREAK	
13:30 - 14:30	SATELLITE SYMPOSIUM BD LATEST TECHNOLOGIES AND INNOVATIONS	BD SPONSORED GHIDA SLEIMAN, BD JENS FLEISCHER, BD
14:30 - 16:00	SESSION 1: INNATE IMMUNE SYSTEM	Chairs: DUYGU SAG, KEN J ISHII
14:30 - 15:00	NUCLEIC ACID-BASED IMMUNE-PROPHYLAXIS AND-THERAPEUTICS	KEN J. ISHII (JPN)
15:00 - 15:30	A SYSTEMS BIOLOGY APPROACH TO ASSESS HUMAN NASAL MUCOSAL IMMUNE RESPONSES	JAMES DI SANTO (FRA) (ONLINE)
15:30 - 15:45	STRONG T CELL-MEDIATED ANTITUMOR IMMUNE RESPONSE BY DELIVERY OF PLGA ENCAPSULATED STING AGONIST	EBRU NURAY (TUR)
15:45 - 16:00	IMPACT OF RETINOIC ACID ON GROUP 2 INNATE LYMPHOID CELL PLASTICITY IN PATIENTS WITH RELAPSING-REMITTING MULTIPLE SCLEROSIS	ALTUG OZKOSAR (TUR)
16:00 - 16:30	COFFEE BREAK	
16:30 - 18:00	SESSION 2: COVID-19 AND FUTURE PANDEMICS: ROAD AHEAD	Chairs: ARZU ARAL, AYCA SAYI YAZGAN
16:30 - 17:00	FUNCTIONAL STATUS OF T CELL RESPONSES TO SARS-COV-2: DISEASE VS WHOLE-VIRION VACCINATION	GUNES ESENDAGLI (TUR)
17:00 - 17:30	SARS-COV2 SUPERANTIGENIC AND NEUROTOXIN LIKE MOTIFS IN SPIKE PROTEIN AND POST COVID SYNDROMES	MOSHE ARDITI (USA)
17:30 - 17:45	CORRELATION OF CD68B+MONOCYTES / POLYMORPHONUCLEAR MYELOID-DERIVED SUPPRESSOR CELLS (PMN-MDSC) RATIO AND THEIR EFFECT ON COVID-19 PROGRESSION	DIGDEM YOYEN ERMIS (TUR)
17:45 - 18:00	IMMUNOMODULATORY EFFECTS OF SARS-COV-2 ENCODED PROTEINS	YAGMUR ATALAY (TUR)
18:00 - 20:30	POSTER SESSIONS	

FRIDAY, OCTOBER 21st, 2022

08:00 - 09:30	SESSION 3: IMMUNE SYSTEM DISORDERS: CHALLENGES AND OPPORTUNITIES	Chairs: MAYDA GURSEL, GUNNUR DENİZ
08:00 - 08:30	MODELING LRBA DEFICIENCY	BATU ERMAN (TUR)
08:30 - 09:00	GENETIC SUSCEPTIBILITY TO VIRAL ENCEPHALITIS IN HUMANS	SERKAN BELKAYA (TUR)
09:00 - 09:15	CD20 EXPRESSING LYMPHOCYTES ARE A DISTINGUISHED POPULATION OF CELLS WITH SUPERIOR CYTOTOXIC AND MEMORY FUNCTION AND HIGHLY ENRICHED IN BLOOD AND TISSUES OF PATIENTS WITH AUTOIMMUNITY AND CANCER	OZGUR ALBAYRAK (TUR)
09:15 - 09:30	SILENCING KCNS3 AND FCRLB GENES IN THE EXPERIMENTAL MODEL OF MUSK MYASTHENIA GRAVIS: TWO NOVEL MOLECULES IN MG PATHOGENESIS	GIZEM KORAL (TUR)
09:30 - 10:00	COFFEE BREAK	
10:00 - 11:30	SESSION 4: NOVEL THERAPEUTIC TARGETS IN CANCER	Chairs: TOLGA SUTLU, GERHARD WINGENDER
10:00 - 10:30	IMMUNE RELATED OXIDATIVE STRESS BIOMARKERS IN TUMOR IMMUNE MICROENVIRONMENT TO PREDICT IMMUNOTHERAPY RESPONSE	SUHENDAN EKMEKÇIOĞLU (USA)
10:30 - 11:00	APPLICATIONS OF AI BASED MULTIOMICS DATA ANALYSIS IN IMMUNE RELATED DISEASES	UGUR SEZERMAN (TUR)
11:00 - 11:30	MYELOID-DERIVED SUPPRESSOR CELL HETEROGENEITY INFORMS IMMUNOTHERAPY OF GLIOBLASTOMA	DEFNE BAYIK WATSON (USA) (ONLINE)
11:30 - 13:00	SESSION 5: CANCER IMMUNE THERAPY: RECENT PROSPECTS	Chairs: GUHER SARUHAN DİREŞKENELİ, KEN J ISHII
11:30 - 12:00	THE ROLES OF NONCODING RNAs IN TUMOR IMMUNITY AND EXAMINATION OF HIGH THROUGHPUT CANCER DATA THROUGH BIOINFORMATIC APPROACHES	H. ATAKAN EKİZ (TUR)
12:00 - 12:30	DEATH RECEPTOR SIGNALING IN THE TUMOR MICROENVIRONMENT: WHAT DOESN'T KILL YOU MAKES YOU A FIGHTER	DUYGU SAG (TUR)
12:30 - 12:45	MODULATION OF TCR-NK CELL ACTIVITY BY THE CD8 CO-RECEPTOR AND KIR2DL1	ZEYNEP SENA KARAHAN (TUR)

12:45 - 13:00	FOLATE RECEPTOR-B IS A MARKER FOR MACROPHAGES IN TUMOR-BEARING ANIMALS	SIBEL GOKSEN (TUR)
13:00 - 13:30	LUNCH BREAK	
13:30 - 14:30	SATELLITE SYMPOSIUM	GEN-ERA SPONSORED
	SINGLE-CELL SEQUENCING IN IMMUNOLOGY RESEARCH	TOLGA SUTLU (TUR)
14:30 - 16:00	SESSION 6: IMMUNITY TO INFECTION	Chairs: MEHMET ALI OKTEM, BARBAROS ORAL
14:30 - 15:00	STERILE IMMUNITY AGAINST MALARIA: WHERE ARE WE?	CEVAYIR COBAN (JPN)
15:00 - 15:30	METABOLIC REPROGRAMMING OF BACTERIALLY ACTIVATED B CELLS	AYCA SAYI YAZGAN (TUR)
15:30 - 15:45	VIRAL QUANTITY MATTERS IN INNATE IMMUNE RESPONSE	BANU BAYYURT
15:45 - 16:00	ROLE OF THE CYCLIC DINUCLEOTIDES AND DMXAA DERIVATIVES IN STING-ASSOCIATED AUTOINFLAMMATION	BURCU TEMIZOZ (JPN)
16:00 - 16:30	COFFEE BREAK	
16:30 - 18:00	SESSION 7: EXOSOMES IN HEALTH AND DISEASE	Chairs: BARBAROS ORAL, SERMIN GENC
16:30 - 17:00	BACTERIAL EXTRACELLULAR VESICLES REPROGRAM THE BRAIN TUMOR IMMUNE MICROENVIRONMENT	DIONYSIOS C. WATSON (USA) (ONLINE)
17:00 - 17:30	NEURON-MICROGLIA INTERACTION WITH EXOSOMAL MIRNAS	SERMIN GENC (TUR)
17:30 - 17:45	RUXOLITINIB REGULATES THE EXPRESSION OF TOLL-LIKE RECEPTOR SIGNALING PATHWAY IN CHRONIC MYELOID LEUKEMIA CELLS	LEILA SABOUR TAKANLOU (TUR)
17:45 - 18:00	DENDRITIC CELLS MODULATION BY MESENCHYMAL STEM CELL PROMISES A PROTECTIVE MICROENVIRONMENT AT THE FETO-MATERNAL INTERFACE: IMPROVED OUTCOME OF PREGNANCY IN ABORTION PRONE MICE	MARYAM ESKANDARIAN (IR)
18:00 - 19:30	EFIS SPECIAL LECTURE	Chair: IHSAN GURSEL
18:00 - 19:00	PLENARY LECTURE 1: DISSECTING HUMAN ANTIBODY RESPONSES: USEFUL, BASIC AND SURPRISING FINDINGS	ANTONIO LANZAVECCHIA (ITA) (ONLINE)
19:00 - 19:15	ANALYSIS OF HELPER (TH) AND CYTOTOXIC T CELL (TC) SUBSETS IN MIS-C	ABDURRAHMAN SIMSEK (TUR)
19:15 - 19:30	P.R31* LOSS OF FUNCTION MUTATION IN DIAPH1 OR ITS SILENCING WITH SHRNA IN VITRO RESULTS IN FUNCTIONAL DEFECTS IN T AND NATURAL KILLER CELLS	AHMET EKEN (TUR)
19:30 - 21:30	TSI GENERAL ASSEMBLY MEETING	
SATURDAY, OCTOBER 22nd, 2022		
09:00 - 10:30	SESSION 8: NOVEL TECHNOLOGIES ON IMMUNOLOGY	Chairs: UGUR SEZERMAN, BATU ERMAN
09:00 - 09:30	3D BREAST TUMOR MODELS FOR CANCER IMMUNOTHERAPY	IBRAHIM TARIK OZBOLAT (TUR)
09:30 - 10:00	THE SECRETOME CARGO OF HUMAN MSCS: PLAYERS IN INTERCELLULAR COMMUNICATION AND IMMUNOMODULATION	JOANA MIRANDA (POR)
10:00 - 10:15	TLR9 (TOLL-LIKE RECEPTOR 9) LIGAND SEQUESTRATION ABROGATES CENTRAL B CELL TOLERANCE	ELIF CAKAN (TUR)
10:15 - 10:30	SINGLE-CELL RNA SEQUENCING OF ANTIGEN-SPECIFIC B CELLS FROM COVID-19 VACCINATED INDIVIDUALS ENABLES THE DISCOVERY OF NOVEL ANTIBODIES SEQUENCES AGAINST THE SARS-COV-2 SPIKE PROTEIN	ELIF CELIK (TUR)
10:30 - 10:45	COFFEE BREAK	
10:45 - 11:00	CONGRESS PHOTO SHOOT	
11:00 - 12:00	TURKISH SOCIETY OF IMMUNOLOGY ISIL BERAT BARLAN AWARD CEREMONY	Chairs: CEVAYIR COBAN, GUHER SARUHAN DIRESKENELI
11:00 - 11:15	AWARD PRESENTATION AND SHORT TALK#1 - 12 mins and 3 mins Q&A	
11:15 - 11:30	AWARD PRESENTATION AND SHORT TALK#2 - 12 mins and 3 mins Q&A	
11:30 - 11:45	AWARD PRESENTATION AND SHORT TALK#3 - 12 mins and 3 mins Q&A	
11:45 - 12:00	CLOSING CEREMONY	

ORAL PRESENTATIONS

[OP-01]

Strong T Cell-Mediated Antitumor Immune Response by Delivery of PLGA Encapsulated STING Agonist

Ebru Nur Ay¹, Yusuf Dolen², Eric Van Dinther², Marjolein Schluck², Carl Figdor²

¹Istinye University

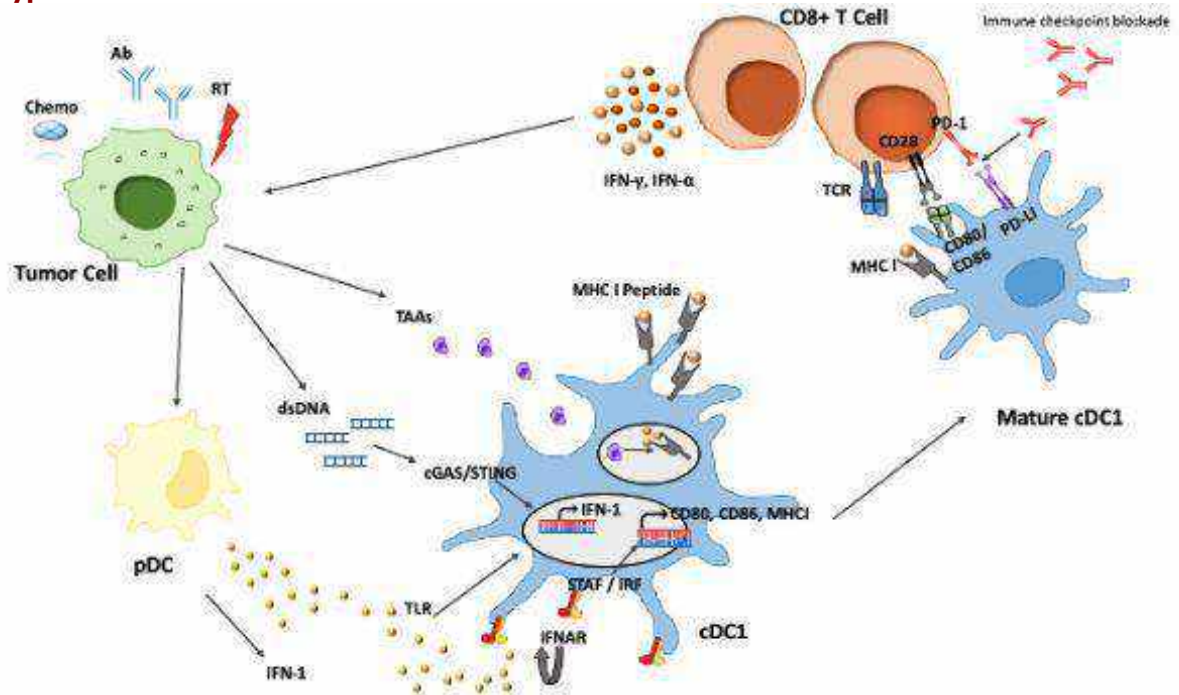
²Radboud University

Synthetic particulate vaccines targeting the activation of the immune system are promising for successful immunotherapy approaches with their advantages such as fewer side effects and tumor specificity compared to conventional cancer treatments. It has been shown that dendritic cells (DCs) potentiate the antitumor response through biodegradable vaccine carriers of immunostimulatory agents, along with the STimulator of INterferon Genes (STING) pathway stimulators in the tumor microenvironment, resulting in a potent antitumor immune response. The main purpose of this research is to develop an effective immunotherapy strategy in cancer treatment with a nanoparticle-based vaccine formulation that can provide a strong immune response. In this study, the effects of the synergistic effect created with STING and Toll-like receptor (TLR) agonists on the therapeutic responses of T cells were investigated by increasing the antigen-presenting capacity of DCs.

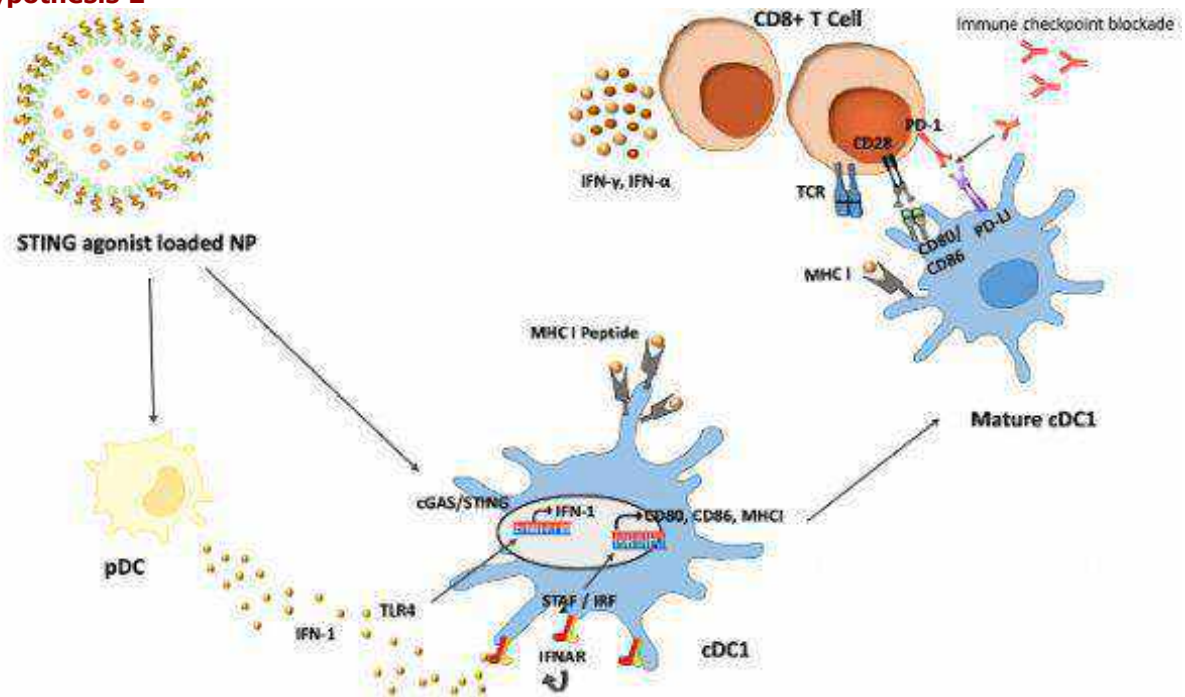
Steady-state classical dendritic cells 1 (cDC1) are known not to respond directly to STING agonists by producing type I IFN (interferon). Therefore, cDCs must first be sensitized either via TLR signaling with (TLR) agonist or with type I IFN produced by another DC subsets. In this study, soluble trials of STING agonists (cGAMP, diAMP) and TLR agonist (CpG) combinations targeting strong T cell activation were planned for type I IFN production from DCs. STING, TLR agonists and tumor antigen ovalbumin (OVA) were also applied to cells in a nanoparticle formulation by encapsulating PLGA (polylactic-co-glycolic acid). Cell stimulation, cytokine assay and T cell proliferation experiments were performed in vitro. According to the results, we have shown that soluble agonist combinations have type I IFN cytokine production and T cell proliferation in DC subsets (CD103⁺, LY-6D⁺). However, it hasn't been seen the same effect in PLGA encapsulated agonists. In future studies, the encapsulation method will be changed in order to obtain a more effective nanoparticle formulation, and the research will continue.

Keywords: Cancer Immunotherapy, DC vaccination, STING pathway, TLR agonist CpG, Type I IFN

Hypothesis 1



Hypothesis 2



[OP-02]

Impact of Retinoic Acid On Group 2 Innate Lymphoid Cell Plasticity In Patients with Relapsing-Remitting Multiple Sclerosis

Altuğ Özkoşar¹, Fatma Betul Oktelik², Metin Yusuf Gelmez², Sevda Öztürk Erden³, Tuncay Gunduz³, Murat Kurtuncu³, Günnur Deniz², Suzan Cinar²

¹Institute of Graduate Studies in Health Sciences, Istanbul University, Istanbul, Türkiye

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Objective: Multiple sclerosis (MS) is an immune-mediated neurodegenerative disease of the central nervous system (CNS). Innate lymphoid cells (ILC1-3) are CD4⁺ T cell counterparts involved in tissue homeostasis and regulation of immune responses. ILCs adapt to local environmental cues by changing their profiles and transdifferentiating into one another via plasticity, which enables them to change their functions. Recent studies showed that IL-33, IL-2, and vitamin A metabolite retinoic acid (RA) are the key factors to induce IL-10 production in group 2 ILCs (ILC2) that are divided into two functionally distinct subsets based on the expression of c-Kit. This study aimed to evaluate the ILC2 diversity, fine-tuned by RA and implicated in CNS inflammation in patients with relapsing-remitting MS (RRMS).

Materials-Methods: Intracellular IL-10 content, the expression of c-Kit and chemokine receptor CCR6 among peripheral blood ILC subsets of untreated RRMS patients (n=11) with a mean age of 40,7 and EDSS of 2,15 and healthy donors (n=10) were analyzed by multicolor flow cytometry after IL-2 and IL-33 with or without RA stimulation for two days.

Results: ILC3 subset was increased in patients with RRMS without any stimulation. In vitro response to IL-2, IL-33, and RA, ILC1 subset was also increased. In contrast, ILC3 cells were diminished in patients with RRMS. Upregulation of IL-10 was impaired in ILC2, c-Kit-ILC2 and ILC3 cell subsets, while IL-10⁺ ILC1 subset were increased and CCR6 expression on ILC1 cells was reduced in RRMS patients.

Conclusions: Advances in treating MS depend on understanding the full range of immunological perturbations, focusing on the critical roles of ILCs in modifying the disease progression. Thus, our findings indicate that, although the relative percentage of IL-10⁺ ILC subsets in RRMS patients was increased, the IL-10 expression remained low and could not be effectively induced by RA in indicated conditions.

Keywords: Retinoic Acid, Innate Lymphoid Cell plasticity, Multiple Sclerosis, Interleukin-10

Correlation of CD66b+monocytes/polymorphonuclear Myeloid-derived Suppressor Cells (PMN-MDSC) Ratio and Their Effect on COVID-19 Progression

Digdem Yoyen Ermis¹, Fatma Dombaz Ozbey¹, Fatma Dombaz Ozbey², Onur Etgu¹, Onur Etgu², Mehmet Karacay¹, Mehmet Karacay², Gozde Arslan¹, Gozde Arslan², Eren Cagan³, Ali Asan⁴, Bahar Dakiki Korucu¹, Bahar Dakiki Korucu², Bahar Dakiki Korucu⁹, Cenktug Korucu⁸, Muhammed Ali Kızmaz¹, Muhammed Ali Kızmaz², Abdurrahman Şimşek¹, Abdurrahman Şimşek², Emel Yılmaz⁵, Esra Kazak⁵, Ibrahim Ethem Pınar⁶, Salih Haldun Bal⁷, Mert Karaca¹, Vildan Özkocaman⁶, Fahir Özkalemtaş⁶, Emin Halis Akalın⁵, Ferah Budak¹, Haluk Barbaros Oral¹

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³University of Health Sciences, Bursa Yuksek Ihtisas Training and Research Hospital, Department of Pediatric Infectious Diseases, Bursa, Türkiye

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Introduction: CD66b+ monocytes first described in cancer and show proinflammatory characteristics. Although their common marker with PMN-MDSCs is CD66b, CD66b+ monocytes are distinguished phenotypically from these cells by their CD33 or CD14 positivity. In this study, the effect of CD66b+ monocytes and PMN-MDSC interaction on disease prognosis in the pathogenesis of COVID-19 was evaluated for the first time in literature. **Material-Methods:** Leukocytes were obtained from 1.077g/mL ficoll phase and whole blood of COVID-19 positive children and adults (mild, moderate, severe disease). The surface molecules (CD45, CD11b, CD66b, CD15, CD14, CD33, CD16, HLA-DR, CD114, CD62-L, Lox-1, PD-L1, PD-L2, CD80, CD86, CD25, CD154, CD69) were studied with flow cytometry. Dichlorodihydrofluorescein diacetate (DCFDA) and 4,5-diaminofluorescein diacetate (DAF-2 DA) were used to measure ROS and NO production by myeloid cells, respectively. Carboxyfluorescein succinimidylester (CFSE) for proliferation analysis of CD4+ or CD8+ T cells obtained from healthy donors in the presence of myeloid cells from COVID-19 positive patients. Cytokines from co-cultures were measured with LEGENDplex (IL-4, IL-2, CXCL10 (IP-10), IL-1 β , TNF- α , CCL2 (MCP-1), IL-17A, IL-6, IL-10, IFN- γ , IL-12p70, CXCL8 (IL-8), TGF- β 1).

Results and Discussion: High proportion of CD66b+ PMN-MDSC cells were observed in children diagnosed with COVID-19 (0-13 years old), and CD66b+ monocytes increased while PMN-MDSC decreased in acute phase of disease. Adult patients with severe disease can not modulate CD66b+ monocytes at the beginning of infection. PMN-MDSCs increased very rapidly in acute phase and were the most prominent sub-population in whole blood and 1.077 Ficoll phase from patients diagnosed with severe disease. These results indicate that CD66b+ cells have two-sided effect on immune responses in COVID-19. Especially in acute phase of anti-viral immune

responses,the presence of high levels of pro-inflammatory CD66b+ promote effective immune responses. This study is being supported by The Scientific and Technological Research Council of Turkey (TUBITAK),Project no.120S653

Keywords: COVID-19, PMN-MDSC, CD66b+ monocytes

[OP-04]

Immunomodulatory Effects Of Sars-Cov-2 Encoded Proteins

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²Izmir International Biomedicine and Genome Institute, İzmir, Turkey

OBJECTIVE

Infection with SARS-CoV-2 inhibits early type I IFN response but activates inflammasome signaling and pro-inflammatory cytokine production. To this end, in addition to structural protein Nucleocapsid, different accessory and non-structural proteins' effect on immunomodulation was assessed by using lentiviral gene transduction system.

MATERIALS AND METHODS

For stable protein expression in THP1-Dual cell line, plasmids for the production of lentiviral vectors that provide packaging and envelope protein expression were combined with SARS-CoV-2 protein plasmids. Cell lines stably expressing, NSP9, NSP10, ORF3a, ORF8, Nucleocapsid, and their mock transduced control were then exposed to virus-mimetic pattern recognition receptor ligands or to recombinant IFN- β to measure type I IFN and type-I-dependent ISG15 production, respectively. Furthermore, to evaluate the impact of SARS-CoV-2-related protein expression on NLRP3, NLRC4, AIM2, or non-canonical inflammasome activation were stimulated with corresponding activating ligands. Cellular IL-1 β cytokine production and lactate dehydrogenase enzyme (LDH) release were analyzed as indicators of inflammasome activation and pyroptosis, respectively.

RESULTS

Our results indicate that ORF3a and ORF8 accessory proteins significantly antagonized type I IFN production and ORF3a downregulated ISG15 production. Specifically, ORF8 expression downmodulated IFN production 3- and 2-fold in response to RIG-I and TLR7/8 agonists, respectively. Moreover, NSP9 and N were found to antagonize type I IFN production only when compared to WT, but not to MOCK. Results related to inflammasome activation indicated showed that ORF3a accessory protein significantly increased NLRP3 and NLRC4 inflammasome-mediated IL-1 β production (5- to 10-fold) and LDH release.

CONCLUSION

Our study suggests that ORF3a and ORF8 accessory proteins can be categorized as type I IFN antagonists. Furthermore, ORF3a accessory protein facilitated NLRP3 and NLRC4 inflammasome-mediated IL-1 β production and LDH release, indicating that the ORF3a viroprotein could contribute to the increased inflammatory response in the host.

Keywords: SARS-COV-2, Antiviral Immunity, Inflammasome, Type I IFN Antagonism

[OP-05]

CD20 Expressing Lymphocytes Are A Distinguished Population Of Cells With Superior Cytotoxic And Memory Function And Highly Enriched In Blood And Tissues Of Patients With Autoimmunity And Cancer

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¹Koç University Research Center For Translational Medicine (KUTTAM)

²Koç University School of Medicine

³Bakırköy Mazhar Osman Psychiatric Hospital

⁴Hacettepe University Department of Basic Oncology

⁵Koç University Hospital Department of Thoracic Surgery

⁶Koç University Hospital Department of Pediatric Hepatology

⁷Koç University Hospital Department of Gastroenterology

⁸Ludwig Maximilian University Department of Immunology

⁹Koç University Hospital Department of Neurology

Objective: Here, we describe a novel subset of NK cells that express low levels of CD20. We uncover features of CD56⁺CD20^{dim} cells that are highly relevant in autoimmune disorders and cancer. Furthermore, we show that these features are shared by CD20⁺ CD4⁺ and CD8⁺ T cells.

Materials-Methods: We used flow cytometry, cell sorting, bead arrays, killing assays and stimulation methods to analyze CD20⁺ lymphocyte subgroups in comparison to CD20⁻ cells in patients with multiple sclerosis (MS), autoimmune hepatitis (AH), hepatocellular carcinoma (HCC), lung cancer (LC) and healthy controls. Publicly available single cell RNA sequencing datasets were also used.

Results: We found that 2-25% of NK cells express CD20, being higher in cerebrospinal fluid and liver compared to blood, and are significantly higher in patients with MS, AH, HCC and LC compared to healthy controls. CD20 expressing CD56⁺, in addition to CD8⁺ and CD4⁺ cells degranulate more (increased CD107a⁺ cell ratio and fluorescent intensity) and secrete higher amounts of granzyme A, B, K, perforin, FasL and soluble Fas after stimulation. CD56⁺CD20⁺ cells kill K562 cells more efficiently. CD20⁺ NK cells are enriched in the CD56^{bright} population compared to CD56^{dim}, and CD4 and CD8 cells are enriched in effector memory cells compared to Temra population. Higher ratio of CD8⁺CD20⁺ cells expressed Klrp1, CD57, CD69, HLA-DR, CD161 and PD1. CD127 expression and proliferation capacity was higher in CD20⁺ lymphocytes. Finally, CD20⁺ T cells were more clonally expanded.

Conclusions: We discovered that CD20 is a marker that is found universally on various lymphocyte subsets with higher cytotoxic and memory features. CD20⁺ lymphocytes are enriched in tissues and found significantly higher in diseases characterized by chronic antigenic stimulation. These findings show that CD20⁺ cells can be both the target and means of therapeutic activity in a wide range of disorders.

Keywords: CD20, Lymphocytes, Autoimmunity, Cancer

CD20+ NK Cells in Diseases

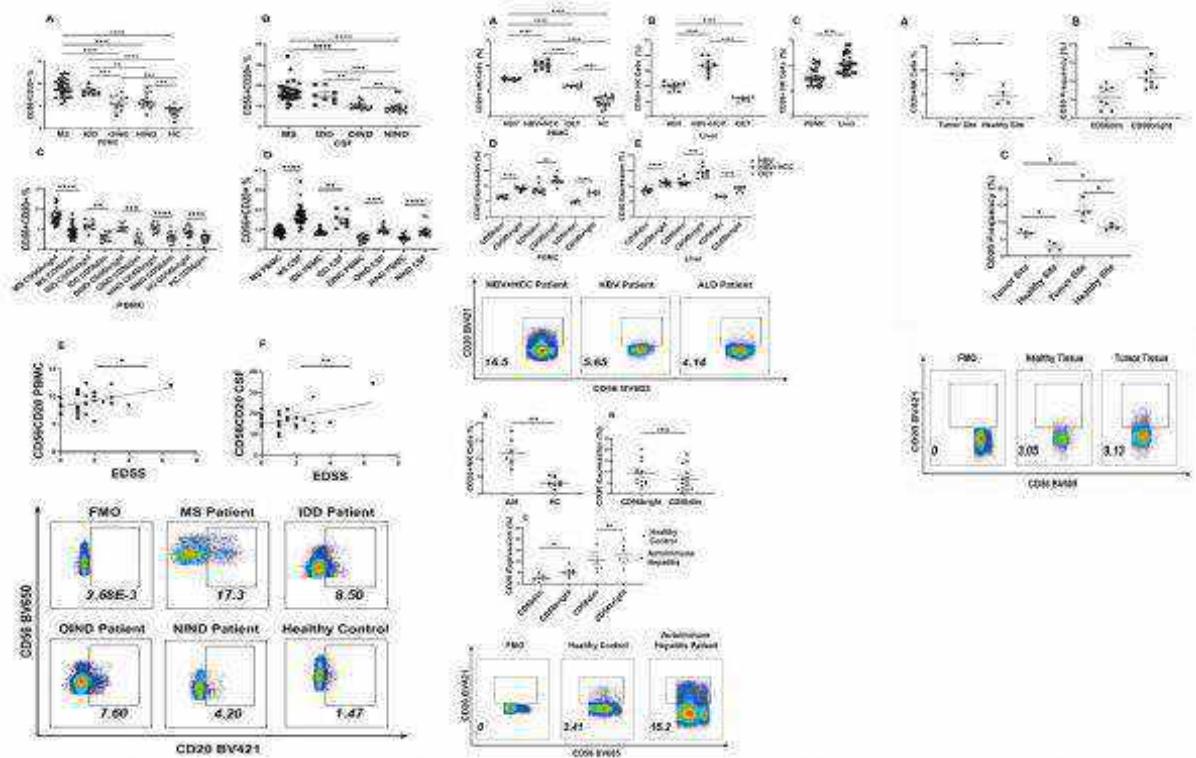


Figure Shows the CD20+ NK Cell Percentages in patients with multiple sclerosis (MS), autoimmune hepatitis (AH), hepatocellular carcinoma (HCC), lung cancer (LC) and healthy controls.

Cytokine And Cytotoxic Molecule Secretion and Killing Capabilities of CD20+ NK Cells

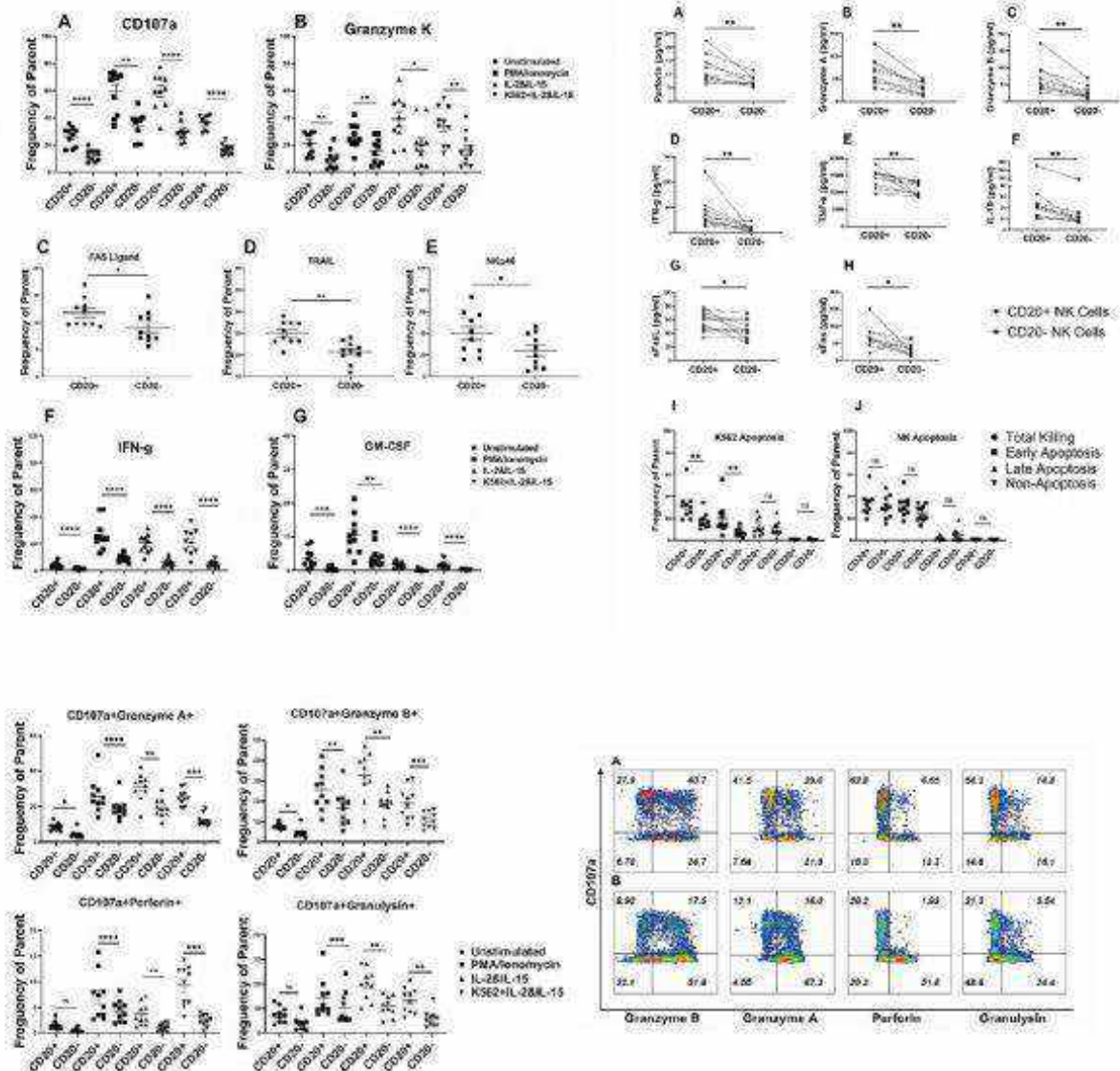


Figure shows the functions of CD20+ NK Cells

[OP-06]

Silencing KCNS3 And FCRLB Genes In The Experimental Model Of Musk Myasthenia Gravis: Two Novel Molecules In MG Pathogenesis

Gizem Koral¹, Canan Aysel Ulusoy¹, Hakan Şahin², Selen Soylu¹, Ece Akbayır¹, Erdem Tüzün¹, Vuslat Yılmaz¹

¹Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Neuroscience

²Istanbul University, Cerrahpasa Faculty of Medicine, Department of Histology and Embryology

Myasthenia gravis (MG) is a prototypic-autoimmune-neuromuscular disease. Muscle-specific-kinase antibodies (MuSK-Abs) in MG reduce neuromuscular transmission through inhibition of MuSK phosphorylation and acetylcholine receptor (AChR) clustering. Our project aimed to determine the effect of silencing FCRLB (Fc Receptor Like B) and KCNS3 (Potassium Voltage-Gated-Channel-Modifier Subfamily-S Member-3) genes using shRNA on the experimental autoimmune MuSK MG.

Materials/Methods

Mice were immunized with 45 µg/ml Ecto-MuSK (6-8 week old, female, 22 Balb/c) in Complete Freund's Adjuvant (CFA). For clinical progression, weight measurement, grip strength test, and clinical scoring were followed. After the second immunization, FCRLB-shRNA, KCNS3-shRNA, Scrambled-shRNA or saline (as control) were injected intraperitoneally. At the end of the experiment serum, skeletal muscle, lymph nodes, and spleen were collected and stored under appropriate conditions. The total amount of anti-MuSK antibody in serum was measured by ELISA. RNA was isolated from splenocytes and expression of FCRLB and KCNS3 genes was validated by RT-qPCR. The muscle samples were labeled with immunofluorescent C3, IgG, and α -bungarotoxin. Immunophenotyping was performed by flow cytometry in the cells from lymph nodes.

Results

Disease severity was significantly higher in KCNS3-gene-silenced mice, compared to the MuSK-MG mice (saline-treated). In the FCRLB-shRNA group, significant amelioration was observed in mice. The amount of MuSK-IgG found in the serum was significantly less in the FCRLB-group ($p<0.0001$). There was also a significant difference between the groups in C3 and IgG deposits at the neuromuscular junction ($p<0,0001$). CD19+CD5+B1a cells were significantly lower in the FCRLB-group.

Conclusions

Silencing FCRLB (associated with autoantibody production) cause amelioration in the EAMG model, while the KCNS3 gene silencing worsen the disease. KCNS3, specifically expressed in muscle, may have functions related to muscle strength and the immune system. It has been revealed that the FCRLB gene may be a treatment target. It was thought that further research on these genes should be done.

Keywords: Myasthenia gravis, FCRLB, KCNS3, experimental autoimmune myasthenia gravis, MuSK

[OP-07]

Modulation of TCR-NK cell activity by the CD8 co-receptor and KIR2DL1

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Objective: The clinical development of genetically modified TCR-T cells has so far been hampered by problems caused by the mispairing of endogenously produced TCR chains with the ectopically delivered ones. Since NK cells do not express any endogenous TCR chains, TCR modification of NK cells stands out as a potential solution to this problem. TCR-NK cells have shown promising results in vitro and in vivo, but their exact signalling mechanisms remain to be studied. CD8 co-receptors are known to aid T cell signalling by increasing the strength of the bond between TCR/CD3 and peptide-major histocompatibility complex (pMHC). On the other hand, Killer-cell immunoglobulin-like receptors (KIRs) are among the most prominent receptors regulating NK cell development and function. This study aims to investigate the possible role of CD8 co-receptors and the inhibitory receptor KIR2DL1 in modifying the activity of TCR-NK cells.

Materials-Methods: Lentiviral constructs containing CD8 α , CD8 β and KIR2DL1 genes were delivered to NK cells expressing a functional TCR complex. Effector functions and antigen-specificity against the target cells were analysed by degranulation, cytokine secretion and cytotoxicity assays. Western blot analysis was performed to observe the signalling pathways affected.

Results: Our results show that while the CD8 $\alpha\beta$ heterodimer, CD8 $\beta\beta$ homodimer and KIR2DL1 positively regulate cytotoxicity of TCR-NK cells, CD8 $\alpha\alpha$ homodimer showed some inhibition of antigen-specific responses in TCR-NK cells. Non-specific activity of TCR-NK cells were also affected which shows that fine tuning of TCR-NK cell activity is possible through additional genetic modifications.

Conclusion: This study demonstrates that TCR-NK cell activity is subject to modulation by co-receptors of T cells as well as inhibitory receptors of NK cells. Further analyses to elucidate the exact intracellular signalling pathways taking part in these responses is ongoing.

Keywords: TCR gene therapy, NK cells, Cancer Immunotherapy, TCR-NK

[OP-08]

Folate receptor- β is a marker for macrophages in tumor-bearing animals

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The immune microenvironment shows heterogeneity at different stages of breast cancer development. It is crucial to have a common marker for targeting and modulation of myeloid cells, the dominant population in the heterogeneous tumour microenvironment. In this study, orthotopic tumor models were created with triple-negative 4T1, EMT6 and EMT6/AR1 breast cancer cells with different drug resistance properties. It aimed to investigate the presence of folate receptor (FR)- β as a myeloid cell marker in the immune microenvironment of the tumor by evaluating the time-dependent change of FR- β expression levels in the myeloid cell compartments infiltrating the tumor and the lung tissue as a target organ for metastatic spread. In addition, the variation of FR- β expression in myeloid cell populations infiltrated into tissues of healthy animals with other tissues of animals with advanced cancer was investigated comparatively. It has been determined that FR isoforms are specific for tumor formation, especially FR- β expression on CD206+ macrophages. Regardless of drug resistance traits, FR- β expression increased in a time-dependent manner in tumor-infiltrating macrophage populations. It was observed that PMN populations were predominant, but numerically CD206+ macrophages increased when myeloid cells infiltrating the tissues of animals with advanced tumor burden were compared to healthy controls. Again, FR- β expression was identified to be a specific marker for CD206+ macrophages and increased compared to healthy controls. Therefore, FR- β may be preferred as a suitable target for modulation of macrophages in the tumor microenvironment.

Keywords: Breast cancer, Myeloid cells, Macrophages, Folate receptor- β

[OP-10]

***Mycobacterium Tuberculosis*-specific Tbet⁺ CD4⁺ memory T cells contribute to trained immunity against cancer and viral infection**

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Clinical studies showed that hot water extract of *Mycobacterium tuberculosis* (*Mtb*) holds anti-tumor and anti-microbial potential, especially against non-*Mtb* pathogens. However, mechanisms mediating its anti-tumor and anti-microbial effects have not been identified, yet. Here, we revealed that hot water extract of *Mtb*, abbreviated as MX, exerted strong anti-tumor effect via the mechanisms regulated by IFN- γ -secreting CD4 T cells and *Mtb*-derived STING agonists found in MX only when mice were immunized with MX or BCG. Yet, administration of a MX-derived *Mtb* antigen, to BCG-immunized mice also inhibited tumor growth. Moreover, *Mtb* purified protein derivative (PPD)-reactive Th1 cells in human PBMCs were also reactive to MX, indicating that these cells could be responsible for the anti-tumor effect of MX in humans. Furthermore, MX could promote SARS-CoV-2-specific T cell responses via modulation of the Treg/Th1 balance in SARS-CoV-2-naïve healthy PBMCs with pre-existing *Mtb* memory. Thus, MX might demonstrate therapeutic potential for both cancer and COVID-19 by training adaptive Th1 responses in individuals with BCG immunization or tuberculosis history.

Keywords: MX, BCG, *Mycobacterium tuberculosis* extract, anti-tumor, anti-viral, Tbet

Ruxolitinib Regulates the Expression of Toll-like Receptor Signaling Pathway in Chronic Myeloid Leukemia Cells

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Background Functional toll-like receptors (TLRs) could modulate/regulate anti-tumor effects by activating the cytotoxic T-cells response and inflammatory cytokines. High levels of TLR expression could promote tumor progression, since TLR-stimulated inflammation might induce cancer cells extension into the microenvironment. Myd88 is involved in activation NF- κ B via TLRs downstream signaling. After stimulation, Myd88 activates the NF- κ B pathway and the mitogen-activated protein kinase (MAPK) pathway by forming a signalling complex that consists of various intermediary proteins, such as IL-1R-associated kinases (IRAKs) and TRAF6. Several hematological malignancies have been linked to abnormal expression or genetic deficiencies in the TLR signaling pathway. Ruxolitinib is the first agent used in myelofibrosis treatment with its potent JAK/STAT inhibitory effect. In this research, we aimed to study the anti-leukemic effect of Ruxolitinib on TLR pathway components in K-562 human chronic myeloid leukemia cell line compared to NCI-BL2171 human healthy B lymphocyte cell line.

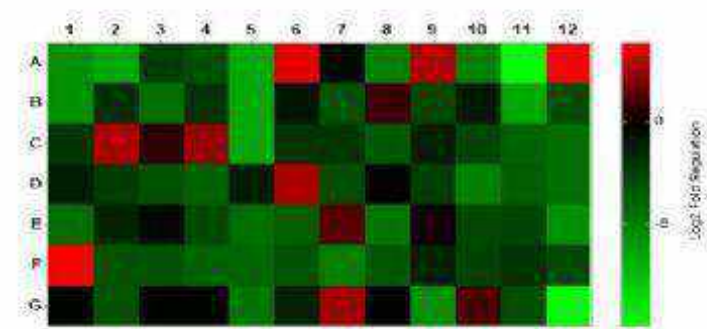
Methods Cytotoxic effect of Ruxolitinib was determined by using WST-1 assay in K-562 and NCI-BL 2171 cell lines. To determine the TLR signaling pathway related gene expression changes, total RNA was isolated from K-562 and NCI-BL2171 cells treated with Ruxolitinib and untreated cells as control group. Reverse transcription procedure was performed for cDNA synthesis, and gene expressions were shown with RT² Profiler™ PCR Array Human Toll-Like Receptor Signaling Pathway by qRT-PCR.

Results IC₅₀ values for K-562 and NCI-BL2171 cell lines were defined as 20 and 23.6 μ M at the 48th hour, respectively. Ruxolitinib treatment caused a notable decrease in expression of CSF2,MAP4K4,TICAM1,PELI1,TLR7,ELK1,IRF1,MAPK8IP3,MYD88,NFKBIL1,TAB1,IRAK4,LY86,SIGIRR,MAPK8,NFRKB,CXCL8,TICAM2,HRAS,SARM1,LTA,RIPK2,TBK1,TLR4,CD14,IFNG,MAP2K4,TRAF6,NFKBIA,TLR2,HSPD1,PRKRA,IRAK2,PTGS2,TLR1,MAP3K7,CCL2,JUN,IKBKB,IL6,FADD, BTK,ECSIT,REL,TNFRSF1A,CHUK,IL2, and TLR10 genes in K-562 cells, compared to control cell line. Ruxolitinib treatment caused a increase in expression of IL10,TLR9,CLEC4E and CD80 genes in K-562 cells, compared to control cell line.

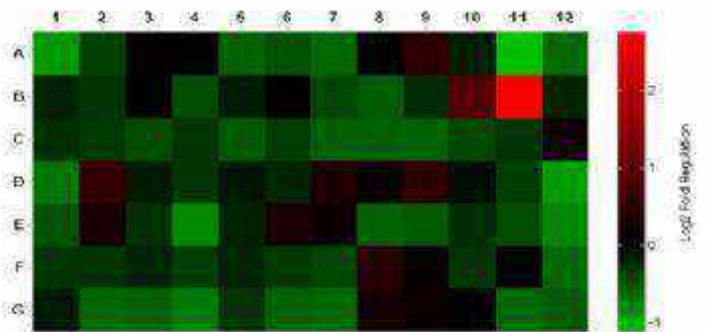
Conclusion Ruxolitinib could be a promising agent in chronic myeloid leukemia treatment by modulating JAK/ STAT and TLR signaling pathway.

Keywords: Toll-like receptor Pathways, Ruxolitinib, Chronic Myeloid Leukemia, JAK/ STAT

Heatmap



TLR signaling pathway's gene expression changes in R562 cell line after oxazolone treatment



TLR signaling pathway's gene expression changes in MCL-DL 2171 cell line after oxazolinic treatment

List of genes

	1	2	3	4	5	6	7	8	9	10	11	12
A	BTK	CASP8	CCL2	CD14	CD180	CD80	CD86	CHUK	CLEC4E	CSF2	CSF3	CXCL10
B	ECSIT	EF2AK2	ELK1	FADD	FOS	HMOX1	HRAS	HSPA1A	HSPD1	IFNA1	IFNB1	IFNG
C	KBKB	IL10	IL12A	IL1A	IL1B	IL2	IL6	CXCL8	IRAK1	IRAK2	IRAK4	IRF1
D	IRF3	JUN	LTA	LY86	LY96	MAP2K3	MAP2K4	MAP3K1	MAP3K7	MAP4K4	MAPK8	MAPK9P3
E	MYD88	NFKB1	NFKB2	NFKBIA	NFKBIL1	NFKB3	NR1C2	PELI1	PPARA	PRERA	PTGS2	REL
F	RELA	RIPK1	SARM1	SIGIRR	TAB1	TBK1	TICAM1	TICAM2	TIRAP	TLR1	TLR10	TLR2
G	TLR3	TLR4	TLR5	TLR6	TLR7	TLR8	TLR9	TNF	TNFRSF1A	TOLLIP	TRAF6	UBE2N
H	ACTB	B2M	GAPDH	HPRT1	RPLP0	HGDC	RTC	RTC	RTC	PPC	PPC	PPC

[OP-12]

Dendritic Cells Modulation By Mesenchymal Stem Cell Promises A Protective Microenvironment At The Feto-Maternal Interface: Improved Outcome Of Pregnancy In Abortion Prone Mice

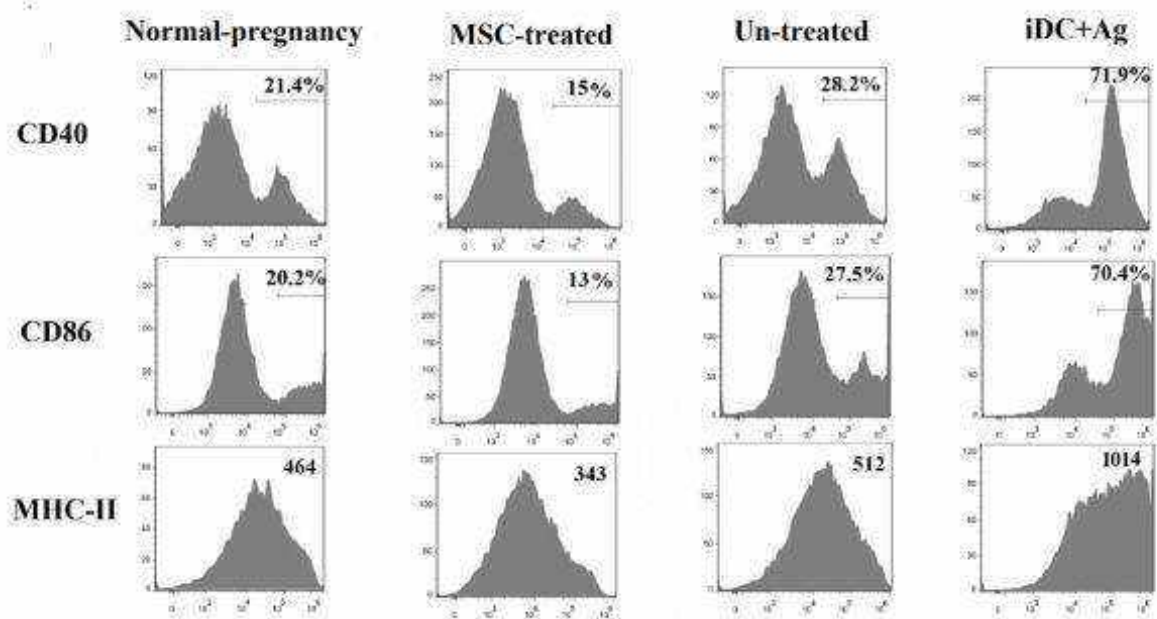
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Objectives: A major fraction of Recurrent spontaneous abortion (RSA) is closely related to disorder of the maternal immune system. MSCs have been shown to exert immunomodulatory effects on immune cells especially dendritic cells. Considering the probable role of dendritic cells in RSA etiology and the immunomodulatory properties of MSC on dendritic cells, we undertook the current study to investigate whether MSCs are capable to modulate the pattern of maternal immune response via the induction of functional changes in decidual DCs, and finally improves the fetal survival and reduces the rate of abortion.

Methods: For this issue, adipose derived mesenchymal stem cells (AD-MSCs) intraperitoneally administered to abortion prone pregnant mice. On day 14.5 of gestation, the number, phenotype and maturation state of decidual dendritic cells were analyzed using flow cytometry. Furthermore, decidual cells from treated and non-treated mice were co-cultured with bone marrow differentiated immature DCs in the presence of paternal antigen. The immunophenotype, antigen uptake properties and the antigen specific T cell stimulation potency of DCs were measured by the flowcytometry analysis. Also, the expression levels of IFN- γ , IL-10, TGF- β , and TNF- α mRNA in dendritic cells also determined by real-time PCR. **Results:** We found that MSCs can induce functional changes on decidual DCs, the expression of MHC-II, CD86 and CD40 markers remarkably decreased on decidual DCs in MSC-treated group. In contrast, CD11b significantly increased in these group as non-treated mice. Also, Our results indicated that decidual cells from MSC- treated group had inhibitory effect on DC maturation and function, a significant reduction in the expression

Keywords: Dendritic cell, Immunology Reproductively, Miscarriage, Microenvironment, Modulation

Effect of decidual cells in fetal site from different source (un-treated, MSC treated and normal pregnancy)on surface phenotype of DCs



[OP-13]

Analysis of helper (Th) and cytotoxic T cell (Tc) subsets in MIS-C

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Aim

Multisystem inflammatory syndrome in children (MIS-C) which appears to be associated to COVID-19 is a severe inflammatory condition that can be observed in various tissues and organs. CD4⁺ and CD8⁺ T cells are divided into different subtypes based on their cytokine production pattern. In this study, we investigated the role of cytokine expressions of CD4⁺T helper, CD8⁺ T cell subsets as well as CD4-CD8⁻ T (double negative, DN) cells in the pathogenesis of MIS-C.

Methods

Peripheral blood mononuclear cells (PBMCs) were extracted by density gradient centrifugation from blood samples of 17 MIS-C patients, 17 pediatric COVID-19 patients, and 17 healthy controls. PBMCs were stimulated with PMA and ionomycin and treated with Brefeldin A in the 4th hour, and a 10-colored Mo Ab panel was evaluated at the end of the 6th hour using flow cytometry.

Results

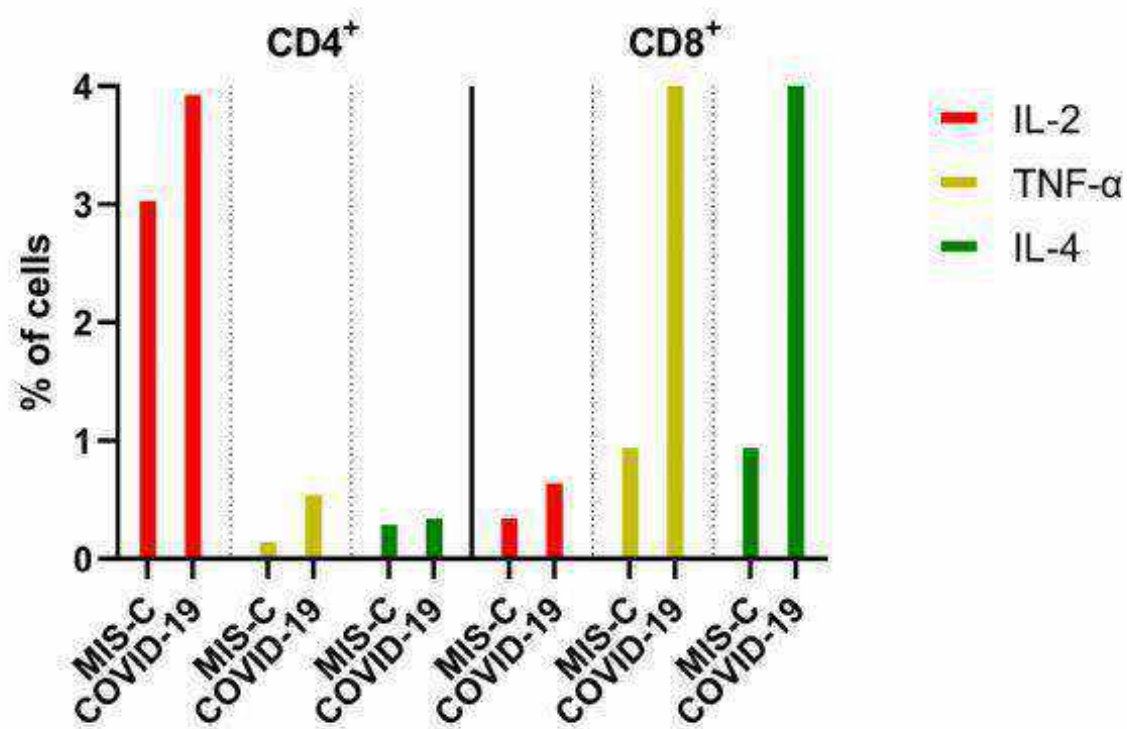
A significant decrease in Tc1 and Tc2 cells was observed in MIS-C cases compared with COVID-19 and the healthy control group. In addition, there was a decreasing trend in Th1 and Th2 cells compared to the same groups. Moreover, Th17 and Tc17 cells increased significantly in MIS-C cases, but there was no difference between COVID-19 and the control group. Interestingly, a significant increase was observed in the MIS-C group in Th17 and Tc17 cells, but not in Th22 and Tc22 cells.

Conclusion and Discussion

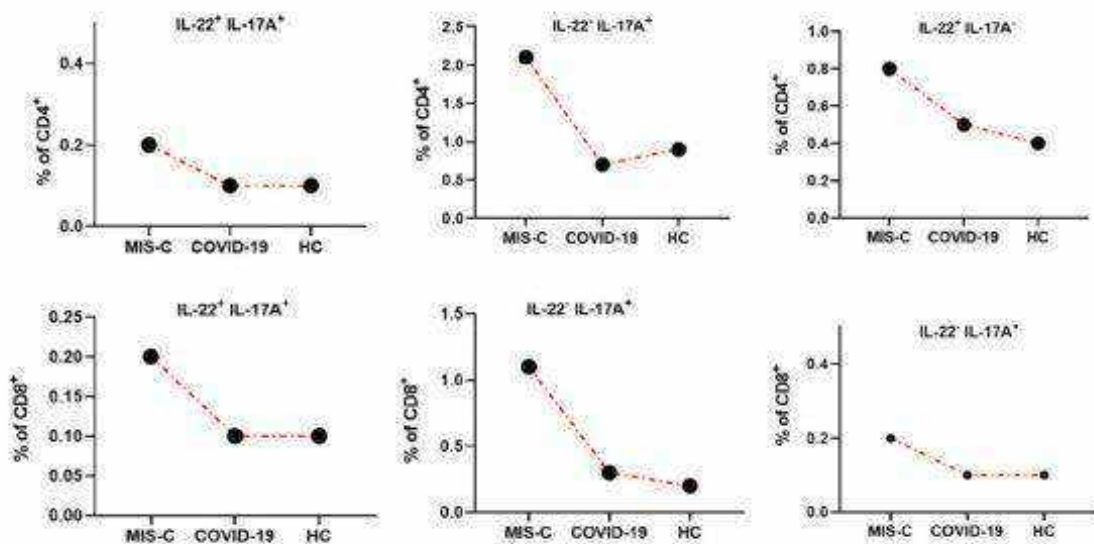
Overall, these results demonstrate a different Th and Tc expression profile in COVID-19 in MIS-C. In particular, the strong increase in Th17 and Tc17 suggests that the key cytokine in MIS-C is IL-17A, as Th22 and Tc22 are not significant compared with COVID-19 and the healthy group. Significant changes in type I and type II cytokines such as IL-2, TNF- α and IL-4 in MIS-C compared with COVID-19 may indicate major differences on Th1,Tc1/Th2,Tc2 axis.

Keywords: MIS-C, COVID19, Tc17, Th17, Tc1, Tc2

Th1,Th2 and Tc1,Tc2 cytokine profiles in COVID-19 and MIS-C



Th17/17 and Th22/Tc22 cytokine profiles between groups



[OP-14]

p.R31* Loss of function mutation in DIAPH1 or its silencing with shRNA in vitro results in functional defects in T and Natural Killer cells

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Background: Diaphanous related formin 1 (DIAPH1) belongs to formin family proteins and is critical in actin polymerization and microtubule stability. Loss of function mutations (LOF) in DIAPH1 are associated with seizures, cortical blindness, and microcephaly syndrome (SCBMS) and are more recently linked to combined immunodeficiency. **Aim:** We aimed to characterize two patients' T and natural killer cells with a LOF mutation in DIAPH1, and the consequences of shRNA-mediated silencing of DIAPH1 in primary T and NK cells.

Methods: Next-generation sequencing of samples from two unrelated patients revealed the same nonsense mutation in DIAPH1 gene, p.R31*. The patients presented with SCBMS and one of them had frequent infections (pneumonia and bronchiolitis), frequent ear drainage, and otitis history. Flow cytometry-based assays, real time qPCR were used for the analyses. **Results:** p.R31* variant led to a significant reduction in the mRNA and protein levels of DIAPH1 in the PBMCs of both patients. DIAPH1-deficient T cells showed proliferation defects in response to both CD3/28 and PHA stimulation in both patients. T cells showed activation defects, characterized by reduced CD69 and CD25 surface expression as well as reduced surface CD3 expression. DIAPH1-deficient PBMCs also showed reduced CD4/CD8 ratio, and impaired transwell migration. DIAPH1-deficient PBMCs showed impaired STAT5 phosphorylation in response to IL-2, IL-7 and IL-15. In addition, the generation of Treg cells from naïve T cells was impaired, although conversion to Treg still occurred, numeric Treg cell expansion greatly diminished. Additionally, NK cells from both patients had diminished cytotoxic activity against K562 cells, reduced granzyme B, TNF- α , and IFN- γ production, and surface CD56, CD94, KLRG1, CD69 and NKp44 expression when cocultured with K562 cells. shRNA-mediated silencing of DIAPH1 reduced DIAPH1 protein level and inhibited T cell proliferation.

Conclusion: Collectively, our data reveal that DIAPH deficiency results in major functional defects in

Keywords: Molecular immunology, NK cells, Adaptive immunity, Immunodeficiency, DIAPH1

Fig 1. p.R31* results in DIAPH1 Deficiency, and major T cell defects

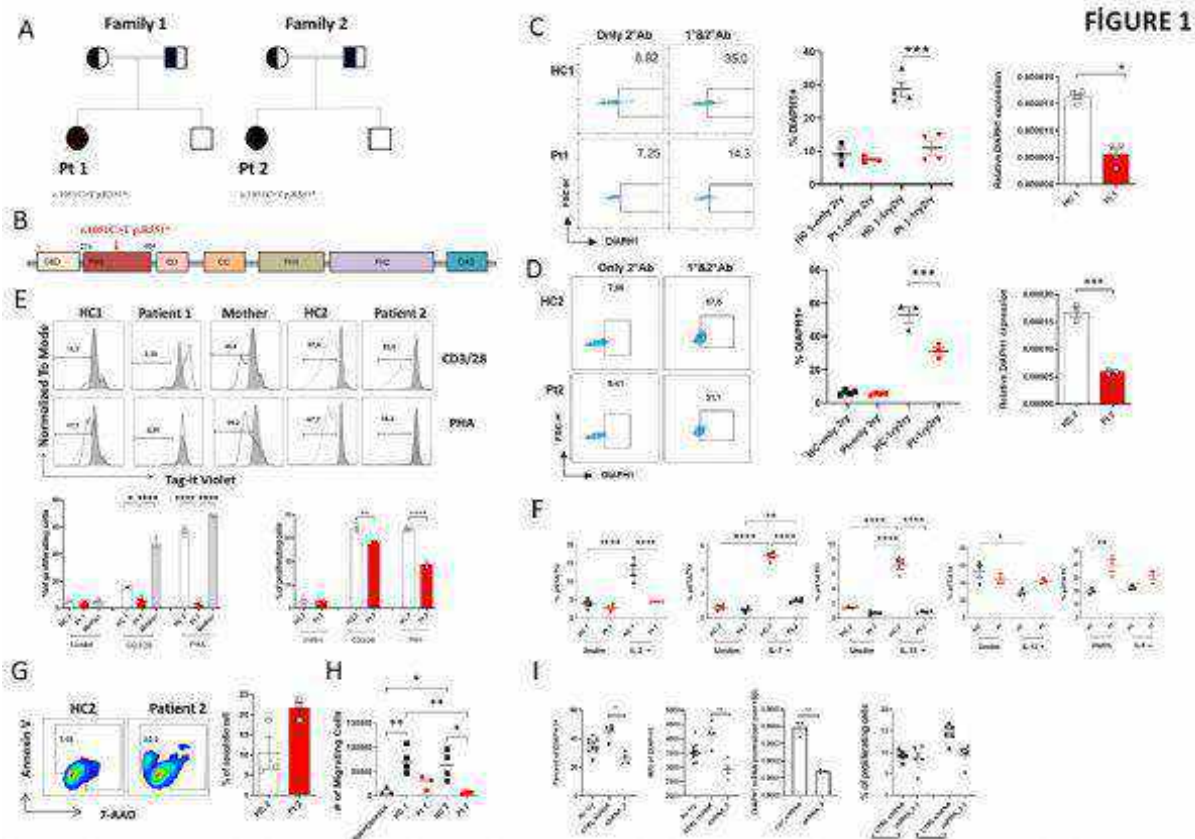
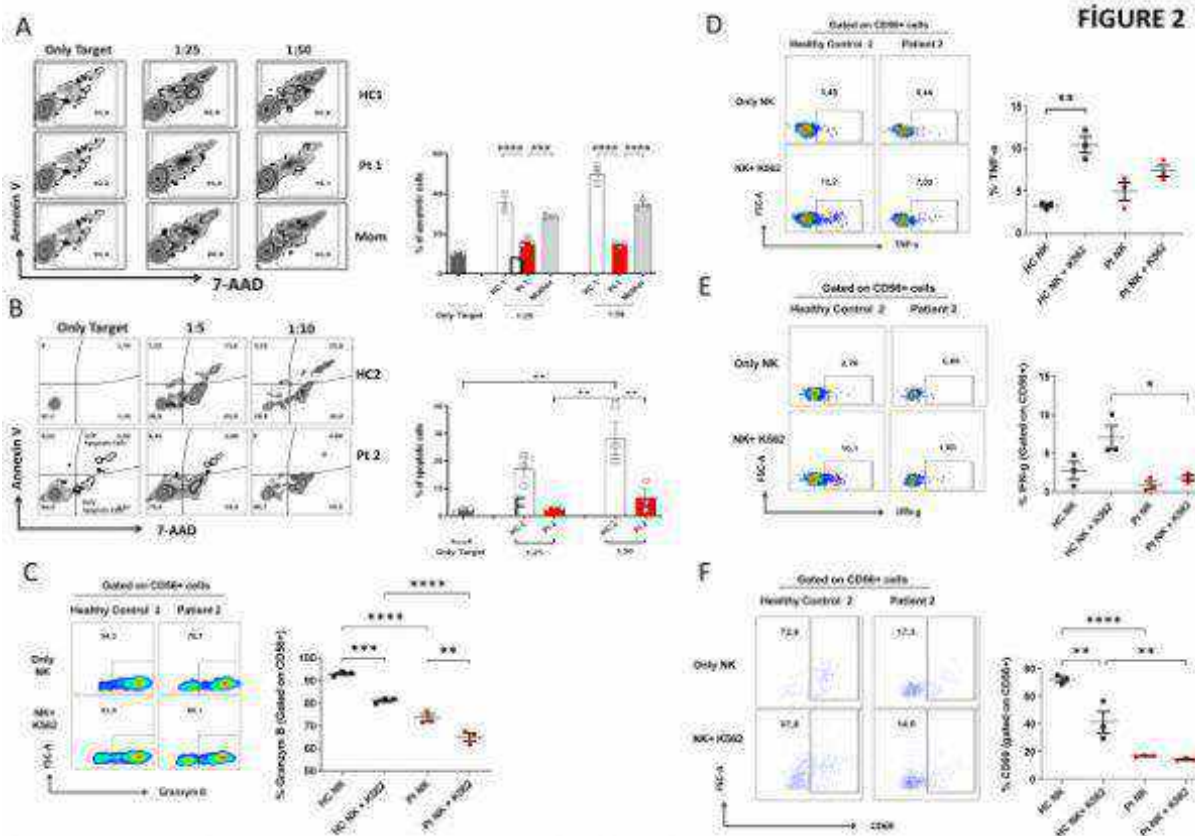


Fig 2. p.R31* results in functional defects in NK cells



TLR9 (Toll-like receptor 9) ligand sequestration abrogates central B cell tolerance

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Objective: Central B cell tolerance is established in the bone marrow and regulated by B-cell receptor (BCR) signaling in immature B cells. Signaling pathways induced by self-antigen recognition by BCR and innate immune receptors are believed to induce the elimination of autoreactive B cell from the repertoire. Defective central B cell tolerance in patients carrying IRAK4, MYD88, TACI mutations and mice injected with hydroxychloroquine shows the involvement of endosomal TLRs but whether TLR7, TLR9 or both is responsible was not known.

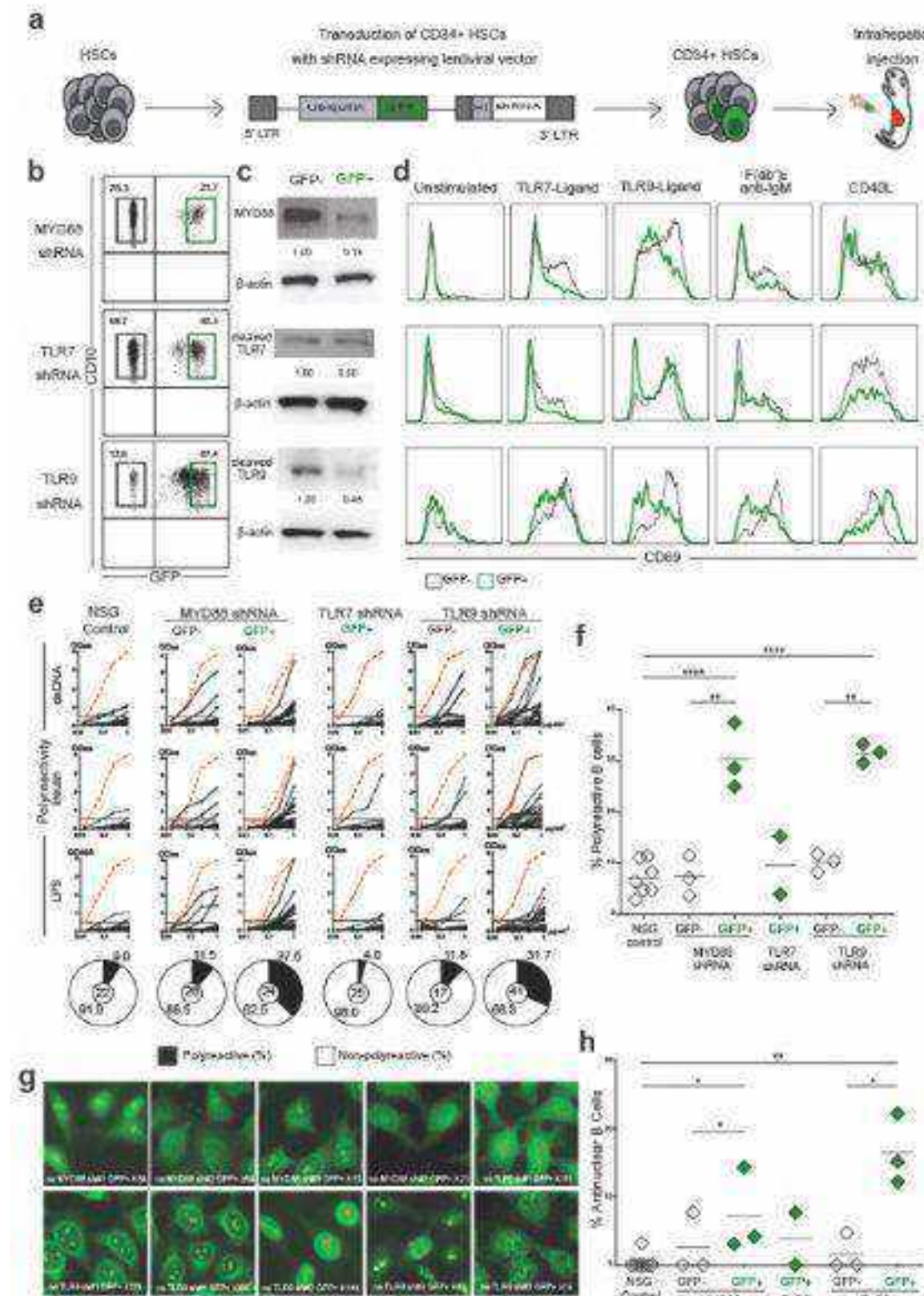
Materials-Methods: CD34⁺ HSCs were injected to NSG mice for humanized mice model. shRNA targeting MYD88, TLR7 and TLR9 were used for the downregulation. All patients

gave informed consent before participation. Single cell cloning was performed to assess the polyreactivity.

Results and Conclusions: Using humanized mice model with downregulated MYD88, TLR7 and TLR9 expression, we observed that MYD88/TLR9 signaling but not the TLR7 is required for the silencing of the autoreactive B cells. We also showed that systemic sclerosis (SSc) patients have defective TLR9 function. CXCL4, a chemokine found to be higher in SSc patients and correlated with the prognosis, is proved to be responsible for the defective TLR9 function by leading to dyslocalization of TLR9 ligand, away from the endosomal compartments. Additionally, defective central B cell tolerance is found in the humanized mice model overexpressing the CXCL4. Also, defective induction of AID after B cell stimulation in the presence of CXCL4 is observed explaining the defective elimination of autoreactive clones from the repertoire. In conclusion, we showed that TLR9 plays an essential role for the regulation of central B cell tolerance and CXCL4 is involved in the pathogenesis of SSc by effecting the TLR9 function leading to a break in central B cell tolerance and secretion of autoantibodies.

Keywords: TLR9, CXCL4, B cells, Systemic Sclerosis

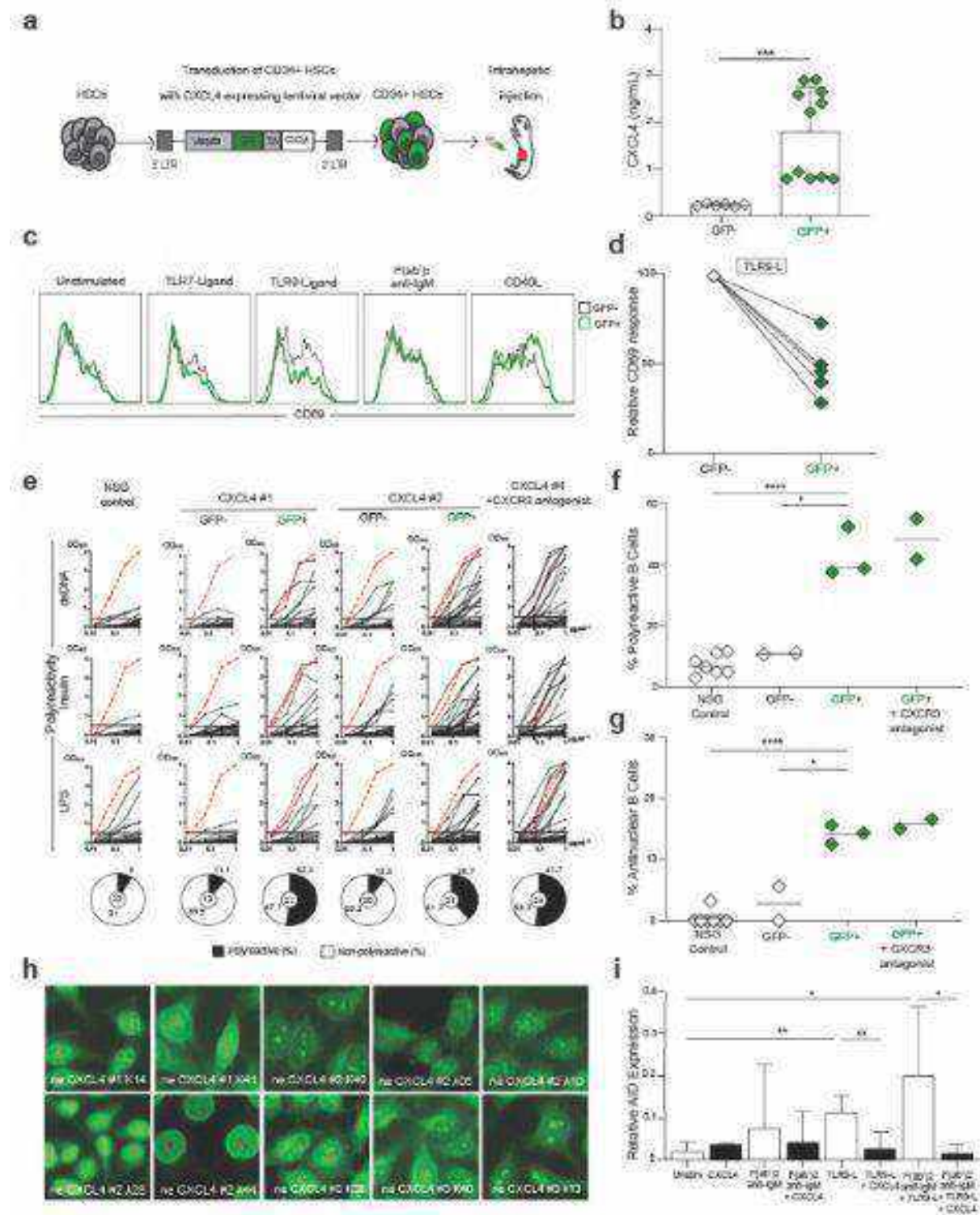
Figure 1. TLR9 is essential for central B cell tolerance



a, Schematic diagram describing the generation of humanized mice. CD34⁺ HSCs transduced with GFP-tagged lentiviruses expressing MYD88, TLR7 or TLR9 shRNA were injected into the liver of 3day-old recipient NSG mice. *b*, Representative flow cytometry analysis showing gating strategy to sort GFP⁻ and GFP⁺ CD19⁺ splenocytes from mice engrafted with HSCs transduced with the indicated GFP-tagged lentiviruses. *c*, Expression analysis of indicated proteins in sorted GFP⁺ and GFP⁻ CD19⁺ splenocytes from humanized mice. β -actin is used for normalization of protein expression. *d*, Representative surface CD69 expression in GFP⁻

(black line) compared to GFP⁺ (green line) CD19⁺ splenocytes after 48 hours in culture unstimulated or activated with the indicated ligands or F(ab)² anti-IgM. e, Representative polyreactivity of antibodies cloned from single new emigrant/transitional B cells isolated from indicated humanized mice was tested by ELISA against dsDNA, insulin and LPS. Dotted red lines show the positive control. Pie charts represent the frequencies of reactive (solid) and non-reactive (open) clones, with the number of clones tested (n) showed in the center. OD405 nm, optical density. Frequencies of polyreactive, f and antinuclear reactive, h clones in new emigrant/transitional B cells. Each data point summarizes the reactivity data from an average of n=21 cloned recombinant antibodies from control NSG (n= 7), MYD88 shRNA (n=3), TLR7 shRNA (n=2) and TLR9 shRNA (n=3) humanized mice. Averages are shown with a bar, and statistically significant differences are indicated (Student's t-test, *p<0.05, **p<0.001, ****p<0.0001). g, Representative nuclear staining patterns for antibodies cloned from new emigrant/transitional B cells.

Figure 2. In vivo CXCL4 expression abrogates central B cell tolerance.



a, Humanized mice were generated with CD34+ HSCs transfected with GFP-tagged lentiviruses expressing human CXCL4 and injected into the 3-day-old NSG mice. *b*, Human CXCL4 concentrations in cell culture supernatants of GFP- (open diamonds) and GFP+ (green diamonds) B cells were determined by ELISA. *c*, Representative CD69 expression in GFP- (black line) and GFP+ (green line) CD19+ cells from humanized mice after 48 hours in culture with no stimulation (Unstimulated) or activated with the indicated ligands or F(ab')₂ anti-IgM. *d*, Relative frequencies of CD69+ cells in sorted GFP+ (green circles) compared to GFP- (open circles) B cells after 48 hours in culture unstimulated (Unstim.) or activation with the indicated TLR9 ligand. *e*, Representative polyreactivity of recombinant antibodies cloned from single

GFP- and GFP+ (expressing CXCL4) new emigrant/transitional B cells from humanized mice injected with CXCR3 antagonist (n=2) or not (n=3) was tested by ELISA against dsDNA, insulin and LPS. Dotted red lines show positive control. OD405, optical density. For each representative fraction, the frequencies of non-polyreactive (open area) and polyreactive (black area) are summarized in pie charts with the total number of clones tested (n) showed in the center. f, polyreactivity, and g, anti-nuclear reactivity frequencies in GFP+ (green diamonds) and GFP- new emigrant/transitional B cells from the indicated humanized mice and control NSG humanized mice (empty diamonds) are summarized on the right. Mean values (with bars) and statistically significant differences are indicated (Student's t-test, * $p < 0.05$, **** $p < 0.0001$). h, Representative nuclear staining patterns for antibodies cloned from new emigrant/transitional B cells. i, Quantitative real-time PCR measures AICDA mRNA transcripts encoding AID in CD19+ bone marrow B cell precursors from humanized mice after 48 hours in culture with no stimulation (Unstim.) or activated with the indicated ligands (Student's t-test, * $p < 0.05$, ** $p < 0.01$).

[OP-16]

Single-Cell RNA Sequencing Of Antigen-Specific B Cells From COVID-19 Vaccinated Individuals Enables The Discovery Of Novel Antibodies Sequences Against The SARS-Cov-2 Spike Protein

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Objective:

Humoral immune responses provide protective immunity against the SARS-CoV-2 virus via production of neutralizing antibodies. Traditionally, the identification neutralizing antibody sequences has been time-consuming since it relied on the vaccination of animal models and establishment of hybridoma cultures. In this study, we aim to use single-cell RNA sequencing for fast discovery of paired heavy- and light chain B cell receptor (BCR) sequences against the Receptor Binding Domain (RBD) of the SARS-CoV-2 Spike protein from antigen-specific B cells of vaccinated individuals.

Materials-Methods: Whole blood samples were collected from 2 individuals that had received three doses of BNT162b2 mRNA vaccine. SARS CoV-2 RBD Specific B cells were positively selected from total B cell pool using recombinant RBD-bound magnetic beads. Obtained cells were processed using 10x Genomics Chromium Next GEM Single Cell 5' and BCR Amplification Kits. The prepared sequencing libraries were sequenced on Illumina NovaSeq M. Bioinformatics analysis was performed using 10x Genomics Cell Ranger. Common sequences were modeled using AlphaFold2 and docking was analyzed by PRISM.

Results: With both donors combined, a total of 18.719 and 11.425 cells were recovered from RBD-specific B cells and total B cells respectively. From the RBD-specific samples 17.500 clonotypes were discovered and of these 4% had more than one cell assigned. In silico models of discovered antibody sequences showed highly effective docking to the RBD domain, indicating a high level of neutralizing activity.

Conclusion: NGS-based discovery of neutralizing antibody sequences is fast and feasible. This study establishes foundations for faster discovery of antibodies against any novel infectious agent.

Acknowledgement and/or disclaimers:

This project was funded by Epsilon Electronics Industry and Trade.

Keywords: Immune Repertoire Profiling, B cell Receptor, COVID-19, single cell immunology, antibody discovery

POSTER PRESENTATIONS

Modulation of Macrophage Polarization by Medicinal Plant *Tinaspora Cordifolia*

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Objective: *Tinospora cordifolia* (*T.cordifolia*) is a plant containing many bioactive phytochemicals. It has several medicinal properties and is used to relieve symptoms of diseases such as cold, pharyngitis, digestive disorders, diabetes, and ulcers among India and China. The bioactive phytochemicals are shown to have the best affinity against SARS-CoV-2 in molecular docking studies. Moreover, there are few studies concerning *T.cordifolia* and macrophages, which were limited to mice and reported conflicting results. However, the effect of *T.cordifolia* on human macrophage polarization has not been investigated.

Materials-Methods: Primary human monocyte-derived macrophages (M0 macrophages), were either left untreated or pretreated with *T.cordifolia* extract at doses of 50 µg/mL, 100 µg/mL, 500 µg/mL, or 1.000 µg/mL for 2 h. Afterwards, macrophages were polarized into M1 (LPS, IFNγ), M2a (IL-4), or M2c (IL-10) macrophages for 22 h. M1 (HLA-DR, CD64, CD86) and M2 (CD200R, CD206, CD163) surface markers were analyzed by flow cytometry. The M1 cytokine TNF was analyzed by ELISA.

Results: In M0 macrophages, *T.cordifolia* extract treatment increased the expression of the M1 marker CD86, while it decreased the expression of the M2a marker CD200R. In M2a and M2c macrophages, *T.cordifolia* extract decreased the expression of the M2a marker CD200R and the M2c marker CD163. Interestingly, after treatment with *T.cordifolia* extract, the phagocytic receptors CD64 and CD206 were upregulated in M0, M1, M2a, and M2c macrophages. Finally, *T.cordifolia* extract treatment enhanced the production of TNF in M0, M2a, and M2c macrophages.

Conclusion: *T.cordifolia* extract shifts the polarization of primary human macrophages into a pro-inflammatory/virus fighting M1 phenotype, with an upregulation of the phagocytic receptors CD64 and CD206. Therefore, *T.cordifolia* may be a potential new supplement for modulation of macrophage polarization for the treatment of infectious diseases, in which macrophages are known to play an important role.

Keywords: Medicinal plants, macrophage polarization, inflammation, infectious diseases, SARS-CoV-2

[PP-02]

Inflammasome Hijacking by Pathogens: New Microbial Effectors and Host Inhibitors

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The survival of host organisms depends on their ability to recognize and eliminate pathogens that infect them. Pathogens, in turn, develop strategies to escape the host immune system and multiply. Upon infection, a battle between microorganisms and the host immune system begins and only the organism that implements an effective escape or defense mechanism at the right time and right place gets rid of the other and survives. Inflammasomes are cytosolic protein complexes of the innate immune system that govern this interaction by activating inflammation through the secretion of IL-1 β and by triggering pyroptosis through Gasdermin D cleavage. In response to this host defense, pathogens employ different strategies to hijack the immune system and as central immune modulators of the cellular fate, inflammasomes are primary targets of several microorganisms. Gram-negative bacteria such as *Salmonella Typhimurium* activate the non-canonical Caspase-4/11 inflammasome through the lipopolysaccharide (LPS) present in their cell wall. SARS-CoV-2 virus triggers the NLRP3 inflammasome by changing the ionic flux of the cell through the viroporin ORF3a. Although increasing evidence underlies the importance of inflammasome modulation by pathogens, the exact pathway mediating this interaction is poorly defined. In this study, we aimed to understand the molecular mechanisms of inflammasome and pathogen interaction. Towards this goal, we screened (i) new microbial effectors that modulate inflammasomes and (ii) host inhibitors that restrict the proliferation of pathogens by using various cellular, molecular, and biochemical techniques including artificial reconstruction of inflammasomes, knock-out of genes and immunostaining. Our results revealed new viral effectors activating inflammasomes and host interferon-stimulating genes restricting pathogen proliferation. Overall, these results bring into light new players of the host/pathogen interaction and will allow the development of new strategies to cure diseases caused by these microorganisms.

Keywords: Inflammasomes, Pyroptosis, Gasdermin, Pathogens, Interferon

PHF6 regulates Notch1 via S199 in T-cell acute lymphoblastic leukemia

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Objective: T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive leukemia distinguished by rapidly proliferation of immature T lymphocytes. One of the key regulator factors of early T-cell differentiation and development is Notch1 and its expression is dependent on PHF6, which defined as a tumor suppressor in T-ALL. In this study, we aimed to evaluate the molecular signaling mechanism of PHF6 by studying the effect of its Serin199 phosphorylation region, which is thought to have a metastatic role in tumor development on protein function in T-ALL cell line DND-41 which have truncated PHF6 expression. Numerous investigations have demonstrated that PHF6 expression is required for Notch1 expression, that inhibiting PHF6 causes Notch1 expression to be reduced or suppressed. Therefore, we wanted to see if PHF6 protein post-translational modification affects its activity.

Materials-Methods: To address this question we first used the PhosphoSite data base to confirm this amino acid was phosphorylated or not and discovered that S199 itself was phosphorylated. Then, we constructed human PHF6 expression vector with pcDNA3.1A, and changed the Serine199 codon to that of non-phosphorylatable Alanine and phosphorylation mimicking Aspartic acid via side-directed mutagenesis, results were confirmed by Sanger sequencing. All vectors transfected to cells via lipofectamine and to examine how these vectors affected Notch1 expression and PHF6 cell proliferation we applied immunoblotting and MTT.

Results: We found that while wild-type PHF6 expression inhibits the proliferation of leukemic cells, this impact is decreased in both Alanine and Aspartic Acid mutants of PHF6. Furthermore, both mutants appear to suppress Notch1 expression, while the Alanine mutant has a stronger impact.

Conclusions: We demonstrate first-ever that S199 phosphorylation-site mutants of PHF6 have altered biological activity, indicating that the signaling pathway inducing phosphorylation of this position may impact PHF6 protein function and Notch1 signalling. That suggests a feedback pathway between these genes.

Keywords: T-cell, acute lymphoblastic leukemia, PHF6, Notch1

The Utility Of CRISPR/Cas9 Technology To Attenuate Apoptotic Pressure in Monoclonal Antibody (Mab) Producing Cells

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Therapeutic monoclonal antibodies (mAbs) are getting wider acceptance in many areas of medicine and biotechnology. The mAbs can be recovered from the medium in which the producer mammalian cells are grown. However, presence of serum in medium complicates purification and isolation of products. Removal of serum causes stress on cells and induces apoptosis. Previous studies have shown that cells lacking the *Bak1* and *Bax* genes (*Bak1*^{-/-} - *Bax*^{-/-}), which initiate apoptosis by causing mitochondrial permeability in cells, do not respond to apoptotic stimuli and grow normally.

In this study, we aimed to permanently inhibit the pro-apoptotic *Bak1* and *Bax* genes of K1 strain of Chinese Hamster Ovary Cells (CHO-K1 cells) with clustered regularly interspaced short palindromic repeats (CRISPR) / CRISPR-associated protein 9 (Cas9) technology and to transfer CHO-K1 cells to serum-free suspension culture for supporting mAb (*Cetuximab_Erbitux*®) production.

The CRISPR/Cas9 system and multiplex single guide RNA (sgRNA) cassette design, which are among the methods were used during this study, was applied to CHO-K1 cells for the first time and the method was utilized for the purpose of knocking out pro-apoptotic genes. CHO-K1 *Bak1*^{-/-} - *Bax*^{-/-} cells were able to produce approximately 10 times more functional mAbs in suspension cultures compared to un-modified parents. These studies demonstrate that attenuation of apoptotic pressure on mAb producing cells contributes significantly to the production of mAbs and CRISPR-Cas9 technology could be a vessel to reach this goal.

Keywords: Monoclonal Antibody, CHO-K1 Cell Line, CRISPR/Cas9, Suspension Culture, Serum-Free Media Adaptation, *Cetuximab_Erbitux*®

Expression, Purification And Immunogenic Characterization Of Multicopy Recombinant Hepatitis B Surface Antigen In Methylophilic *Pichia Pastoris*

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Hepatitis B virus (HBV) is considered as one of the most important blood-borne infectious agent in the world. Despite successful vaccines and antiviral agents, the global threat from chronic hepatitis B infection still continues. Although various successful treatment protocols were developed with antiviral agents, vaccines are still the most effective prevention method against HBV. Vaccines currently available for HBV are second-generation vaccines containing recombinant Hepatitis B surface antigen produced in yeast hosts such as *Saccharomyces cerevisiae* and *Pichia pastoris*. The most preferred method to increase protein production in yeast is to increase the number of copies of the foreign gene to create changes in the yeast genome.

The aim of this study was to produce recombinant HBsAg in *P. pastoris* containing multiple copies of HBsAg gene. To reach high protein concentrations and to maintain the native antigenic properties of the produced protein were the other goals of the study. For this purpose, the foreign gene encoding HBsAg was successfully cloned into the pPICZαA plasmid containing the AOX1 promoter, first as one copy, then two and finally as eight copies. After culturing and methanol induction, high amounts of antigenic HBsAg were detected in the cells. The culture supernatants were removed and the yeast cells were disrupted under high pressure and the resulting suspension was subjected to various processes including purification, absorption and chromatography. The antigenicity of rHBsAg were compared with the commercially available HBV vaccine (EngerixB®) in western blot and enzyme immunoassays using immunized serum from humans and experimental animals. According to the results of these immunological and biochemical assays, rHBsAg produced at our laboratory were very similar to the commercially available vaccine in terms of antigenicity and biochemical features.

Keywords: Hepatitis B, HBsAg, Vaccine, Multicopy, Methylophilic, *Pichia pastoris*

miRNA Regulated FoxO Signaling in CD8⁺ T Cells in Triple Negative Breast Cancer Mouse Model

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Objective: Triple negative breast cancer is the most aggressive subtype of all breast cancers. miRNAs are not only key regulators for cancer cells but also for immune cells. Even though numerous studies have elucidated regulatory roles of miRNAs in breast cancer cells, miRNA profiles in CD8⁺ T cells in breast cancer are not yet fully known. Considering that many CD8⁺ T cell functions are influenced by miRNAs, we performed miRNA expression profiling to identify novel relations between immune system and breast cancer.

Materials-Methods: Allograft breast cancer model was established with 4T1 cell and Balb/c mice. Spleen-derived CD8⁺ T cells were isolated from tumor-bearing and control mice. Following RNA isolation, miRNA microarray analysis was performed. Differentially expressed (DE) miRNAs were determined by BRB-array tool. Online target prediction (miRWalk 3.0) and pathway enrichment analysis (WebGestalt-KEGG) tools were used to gain further bioinformatical insight into the functional roles of miRNAs.

Results: Microarray analysis indicated that 41 miRNAs were DE in CD8⁺ T cells from tumor-bearing mice compared to control group ($p < 0.05$). Among DE miRNAs, only 2 of them (mmu-miR-151-5p, mmu-miR-146a-5p) were down-regulated. As a result of pathway enrichment analysis with common targets of these two miRNAs, most significant pathway was FoxO signaling ($p = 3.6181e-7$). Foxo3 which is one of the main molecules in the pathway is a common target for mmu-miR-151-5p and mmu-miR-146a-5p.

Conclusion: Recent studies revealed that Foxo proteins regulate T cell activities. Although there are limited studies about Foxo3, it has been demonstrated that deletion of Foxo3 increases the number of effector and memory CD8⁺ T cells and promotes proliferation. As a result of our study, the decreased expression of mmu-miR-151-5p and mmu-miR-146a-5p in the breast tumor group may regulate T cell function by increasing the expression of Foxo3 gene.

Keywords: Breast Cancer, CD8⁺ T Lymphocyte, miRNA, FoxO Signaling

Intracerebroventricular Injection Of LGI1 Antibody Increase Seizure Susceptibility and Disrupts Cognitive Performance in Autoimmune Encephalitis Rat Model

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Objective: Anti-leucine-rich glioma inactivated 1 (anti-LGI1) encephalitis is a syndrome of immunological etiology characterized by memory deterioration and frequent seizures. Here, we aimed to clarify the role of LGI antibodies in seizure development and cognitive abnormalities in a passive transfer rat model.

Materials-Methods: Purified IgG from anti-LGI encephalitis patients (n=8) and healthy controls (HC) (n=9), and serum physiologic (SF) (n=8) were intracerebroventricularly administered into non-epileptic Wistar rats on consecutive days. Behavioral assessment and EEG recordings were done before and after IgG administration. Then, convulsive dose (45 mg/kg) of pentylenetetrazol (PTZ) was intraperitoneally administered. Seizure stages and acutely induced spike-wave discharges were analyzed.

Results: Latency to motor seizure, first motor seizure duration and latency for myoclonus were increased in LGI1 group compared to HC and SF groups. Additionally, seizure stages were significantly higher in LGI1 antibody given rats ($p<0.05$). Also, vertical activity in open field maze, spontaneous alternation in Y-maze, and discrimination index in novel object recognition test were significantly different in LGI1 group ($p<0.05$).

Conclusion: LGI1 antibodies seem to increase seizure risk and severity as well as impair memory functions. This LGI1 antibody-mediated passive transfer autoimmune encephalitis rat model can be considered as a potential in-vivo model for anti-LGI1 encephalitis. Further studies will enlighten the complex nature of LGI1 antibodies in the epileptogenesis and cognitive deficits observed in anti-LGI1 encephalitis.

Keywords: autoimmune encephalitis, LGI1, autoantibodies, seizures, memory

Effects of *TREM2* Homozygous Mutations (Nasu-Hakola Disease) on Natural Killer Cells

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Objective: Nasu-Hakola disease (NHD) is a rare genetic neurodegenerative disorder characterised by progressive presenile dementia and bone cysts, and leads to precocious death usually during the fifth decade of life. NHD is caused by loss-of-function mutations in *TREM2*, expressed on innate immune cells such as microglia and osteoclasts, involved in the activation of the immune response. In this study, we aimed to analyse the *TREM2* mutation's effect on the peripheral immune system represented by NK cell functions.

Materials-Methods: Five patients with NHD with a defined mutation and 10 age- and gender-matched controls were included in the study. NK cell subsets, KIR expressions (NKG2A, NKG2D, NKp46), cytotoxic activity (CD107a, Perforin, Granzyme A) and cytokine secretions (IL-4, IFN- γ , TNF- α , IL-10, IL-17, TGF- β) were analysed by flow cytometry.

Results: Total NK cell ratio ($p=0.019$), CD3⁺CD56^{dim}CD16⁺ ($p=0.019$) and CD3⁺CD56^{dim}CD16^{dim} ($p=0.04$) subsets were found increased in NHD. Activating C-type lectin receptor NKG2D expression was increased in total ($p=0.005$) as well as CD8⁺ ($p=0.005$) and CD8⁺ ($p=0.003$) NK cell subsets. In total NK cells intracellular TNF- α ($p=0.005$) and TGF- β ($p=0.019$) levels were decreased, IL-17 ($p=0.005$) and IL-4 ($p=0.013$) levels were increased. When NK cells were grouped due to their CD8 expressions IFN- γ and TNF- α levels were decreased and IL-17 were increased in both subsets in the NHD group. Cytotoxic activity showed no differentiation in both unstimulated and K562 stimulated conditions between groups.

Conclusions: In the NHD group, unlike the previous case series, although NK cell ratios and activating receptors such as NKG2D were expressed at high levels, no change in cytotoxic activity was detected. The increased ratio of CD3⁺CD56^{dim}CD16⁺ and CD3⁺CD56^{dim}CD16^{dim} NK cell subsets that overexpress inhibitory checkpoints might explain the low levels of pro-inflammatory cytokines.

Keywords: Nasu-Hakola disease, *TREM2*, natural killer cells, neurodegeneration, cytokines, neuroimmunology

Neutrophil Functions In Patients With A Rare Disease: Pulmonary Alveolar Proteinosis

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Background: Pulmonary alveolar proteinosis (PAP) is a rare interstitial lung disease with a prevalence of 2-6/1.000.000. The inhibition of Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF) caused by blocking auto-antibodies lead to impaired surfactant clearance and could cause fatal respiratory failure and susceptibility for infections. Whole lung lavage performed under general anaesthesia, is only accurate treatment for this serious disease. GM-CSF treatment has been shown beneficial for a subset of PAP patients. The aim of our study is to analyse immunophenotyping and the functional properties of neutrophils in PAP patients.

Method: Eight patients with PAP, and ten age- and gender-matched healthy control subjects were included in this study. After neutrophil isolation from heparinized blood, the cells were incubated in dihydrorhodamine 123. Cells were stimulated with Escherichia coli for phagocytic activity, phorbol myristate acetate for oxidative burst and formyl methionyl-leucyl-phenylalanine for chemotactic activity and analysed by flow cytometry. Index values ≥ 1 , obtained were accepted as normal. Due to aforementioned infection susceptibility the ratio of CD3⁺ T, CD3⁺CD4⁺ T helper, CD3⁺CD8⁺ cytotoxic T, CD3⁺CD19⁺ B and CD3⁺CD16⁺CD56⁺ NK cell ratios were determined by flow cytometry. In addition, demographic data, biochemical parameters and complete blood count were investigated.

Results: Compared to healthy subjects, there were no significant differences in terms of neutrophil count, lymphocyte/neutrophil ratio and neutrophil functions. Renal and liver function tests were normal in PAP patients. Cytotoxic and helper T, B and NK cell percentages were not statistically significant between groups.

Discussion: Neutrophil function tests of PAP patients resulted within normal limits contrary to the literature where it is shown that antimicrobial functions of neutrophils are impaired in patients with PAP. Five out of eight patients had history of GM-CSF treatment which could have affected the neutrophil function tests and might be the underlying reason of the indifference between groups.

Keywords: Pulmonary alveolar proteinosis, GM- CSF, neutrophil migration, oxidative burst, neutrophil phagocytosis

A Metabolic Suppression Model For Th1 Dysfunctionality

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T cell dysfunctionality has been associated with continuous stimulation; however metabolic changes are regulated by the composition of the extracellular milieu is also critical. In order to test the impact of continuously growing cancer cells' metabolites on Th1 cell functions, an in vitro co-culture model was established. PBMCs were isolated from healthy donors by Ficoll separation and CD4⁺ Th cells were enriched by MACS. Then, CD54RA+CCR7⁺ naïve cells were sorted by FACS, co-cultured with THP-1 leukemia cells at increasing ratios. TCR-stimulating aCD3 monoclonal antibody and/or medium were refreshed daily. After 5 days of incubation, proliferation, viability and PD-1, CTLA-4, LAG3, TIM-3 inhibitory receptor upregulation were analyzed. CD4⁺ Th cells' viability and proliferation was decreased as the ratio of leukemia cells increased in the co-cultures. Approximately all Th cells expressed CD45RO. Inhibitory check point receptors CTLA-4, PD-1, TIM-3 and LAG3 percentages increased as the THP-1 ratio increased. In the absence of replacement of leukemia-derived metabolites through medium refreshment, the gradual increase in anti-CD3 antibody concentration did not hinder the upregulation of inhibitor receptors. Their highest levels were observed in the absence of medium refreshment. Our preliminary results indicate the importance of metabolic microenvironment on Th1 cell functions and upregulation of inhibitory receptors.

Keywords: Th1 cell, metabolic suppression, inhibitor receptors

Investigating The Effect Of A Potential Immunomodulatory Protein On Growth Of Colon Cancer “Consensus Molecular Subtype-1 (CMS-1)” Cell Lines

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Personalized cancer therapy is a new approach for planning treatment by considering the genetic makeup, heterogeneity and anti-tumor immune responses against the tumor. Classification of tumors by their molecular characteristics is critical for the development of personalized therapy. Therefore, four different consensus molecular subtypes (CMS) were determined by the molecular characteristics of colon cancer tumors. Our survival analyses using the transcriptome data of colon cancer patients identified a gene within an 11-gene prognostic group correlating with prognosis, within the immune response-associated colon adenocarcinoma molecular subtype (CMS1). The roles of proteins of this gene family have been defined as immune regulation in various cancers, and one of these proteins has also been identified as a novel immunotherapy target. However, the role of the target protein of our study is not yet known on tumor cells or in anti-tumor immune responses. Studies on the function of the protein indicate that it plays a role in neuronal development and is found on T cell microvilli. Our preliminary studies suggest that this transmembrane protein contains an inhibitory ITIM region in its structure, interacting with tyrosine kinases through its cytoplasmic domain, possibly activating a receptor-mediated signaling pathway via an unidentified ligand. Considering this information, we aim to propose the mechanism by which this protein affects the survival of CMS1 group colon cancer patients. In our study, we primarily aimed to examine the effect of altered protein expression on cell proliferation in colon cancer cell lines. Our preliminary results show that protein expression is higher in colon epithelial cell line (CCD-18co) compared to colon cancer cell lines (HT-29, HCT116, LoVo, SW620 and CaCo2). For future studies, we plan to examine the effect of altered protein expression on the growth rate of cells in CMS1 group colon cancer cell lines and propose the mechanism of this effect.

Keywords: colon cancer, CMS1, survival, prognostic biomarker, immune response-associated colon cancer

The Effects Of Polarized Microglia-Derived Exosomes On Neural Progenitor Cell Differentiation

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Microglia are macrophages in the CNS (central nervous system), acting in first line of defense. They are responsible for neural development, including neural differentiation of progenitor cells. Extracellular signals results in polarization of microglia to distinguishing M1 and M2 phenotypes. In this study, we aimed to investigate the distinctive miRNA content of M1 and M2 polarized microglia-derived exosomes and to elucidate the influence of polarized microglial exosomes on neural progenitor cell (NPC) differentiation. The effects of polarized microglia on neural differentiation are assumed to be through exosomal miRNAs; therefore, exosomes were isolated from differentially polarized N9 murine microglia and exosomal miRNA contents were determined via qPCR array. Among mouse immune pathogenic miRNAs, upregulation of 6 miRNAs and downregulation 9 miRNAs have been determined in pro-inflammatory M1 phenotype; while upregulation of 12 miRNAs and downregulation 13 miRNAs have been determined in anti-inflammatory M2 phenotype. To examine in vitro effects of exosomes, we used NPCs differentiated from CGR8 murine embryonic stem cells. NPCs were cultured with polarized microglia derived exosomes in 3D culture. We have demonstrated uptake of exosomes by NPCs, and examined the effects via qPCR and immunocytochemistry methods. Neural markers and also neurite outgrowth of NPCs have been shown to increase upon anti-inflammatory exosome treatment. To further elucidate the effects of inhibition of upregulated miRNAs on NPCs, we used mir134-5p ve mir-34a-5p antogomirs and evaluated the target gene expressions.

The observation of altered neural differentiation through microglial exosomes and their miRNA content is promising to highlight the pathogenesis of several disorders resulting from inflammation and this will eventually provide a new approach in terms of diagnosis and treatment.

This study is supported by TUBITAK (Project no: 218S527)

Keywords: microglia, exosome, neural progenitor cell

Role of Innate and Adaptive Immune System in Patients with Restless Leg Syndrome

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Objective: Restless legs syndrome (RLS) is a sleep disorder that occurs with uncomfortable sensation and an urge to move, especially in the legs at rest. It has been shown that iron/ferritin deficiency, dysregulation of dopaminergic mechanisms and genetic factors play an active role in the disease. In addition, reports show that infectious diseases such as small intestinal bacterial overgrowth and HIV infection increase the prevalence of restless legs syndrome. Obstructive sleep apnea syndrome (OSAS) is one of the most common sleep disorders. Studies conducted with OSAS patients have shown that inflammatory processes associated with OSAS may play a role in the pathophysiology of the disease. The objective of this study was to investigate the relationship between RLS and immune system elements.

Methods: Fourteen RLS patients, 15 RLS patients with OSAS (RLS+OSAS) and 10 healthy subjects were included in the study. T, B, NK, NKT and ILC cell ratio; intracellular IFN- γ , IL-6, IL-10, IL-13 cytokines in T, B, NK cells; CD8⁺ T and NK cell cytotoxic activity were analyzed by flow cytometry. IFN- γ , TNF- α , IL-2, IL-4, IL-6, IL-10, IL-13 levels in plasma samples were evaluated with bead-based soluble molecule assay.

Results and conclusion: Our results showed that compared to the healthy controls the ratio of ILC-1 subset and IL-13+CD4⁺ T cells were increased in RLS and RLS+OSAS patients but the levels of ILC-2 cells were decreased. When the NK cytotoxic activity of RLS patients were evaluated, it was found that the NK perforin levels were lower than OSAS+RLS patients and healthy subjects. Plasma IFN- γ and IL-13 levels were found elevated in the RLS patients with OSAS compared to the healthy subjects and RLS patients. In conclusion, our study revealed that both innate and adaptive immune system elements play a role in RLS.

Keywords: restless legs syndrome, obstructive sleep apnea syndrome, innate lymphoid cells, cytokine, cytotoxicity

Immune- resistance patterns in Acute Myeloid Leukemia

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Aim: To induce immune resistance in acute myeloid leukemia (AML) cells ex-vivo through co-culturing with non-specifically stimulated immune cells and elucidate the characteristics of selected resistant AML sub-population.

Methodology: AML cell lines (THP-1, HL-60, U937) were co-cultured with allogeneic PBMC at 1:0.25 ratio under anti-CD3 stimulation for 48h. The viable AML cells based on DRAQ7 staining and high CD13 expression were purified out through FACS hereafter, referred to as “immune-experienced” AML (ieAML) cells. Morphology (May–Grünwald Giemsa), proliferation (Ki67, cell cycle status and CFSE dilution), and viability (Annexin V/PI) analysis of ieAML cells were performed together with immunophenotyping for myeloid maturation markers by flow cytometry. CD4⁺ and CD8⁺ T cell stimulating (cytokine secretion and proliferation) capacities of ieAML cells were compared with that of wtAML cells in the co-cultures with anti-CD3 antibody. RNA isolated from ieAML cells was subjected to transcriptomics analysis and selected differentially expressed genes were compared with AML patient data retrieved from the TCGA-LAML RNA-seq database. mRNA and protein expression patterns of the targeted genes were confirmed via qRT-PCR and immunofluorescence.

Results: The ieAML cells remained viable with slow paced proliferation along with an increased surface expression of CD80, CD86, CD40, CD14, HLA-DR, PD-L1, CTLA-4 and TIM3 markers and were not negatively influenced even by the highest amount of T cells in terms of viability. CD4⁺ and CD8⁺ T cells proliferation rate reduced in ieAML cells co-cultures. Transcriptomic profile showed 84 common DEG in ieAML cells involved in inflammatory response, anti-apoptosis and cytokine signaling. TCGA analysis showed KLF6, MCL1, B2M and c-MYB overexpression in Poor/Adverse risk groups. Upregulation of KLF6 and downregulation of c-MYB gene was validated through mRNA expression (qRT-PCR) and protein expression (immunofluorescence staining). Our data report the capacity of AML cells to survive strong inflammatory responses and gain additional capacities to cope with anti-tumor immunity.

Keywords: AML- Acute Myeloid Leukemia, PBMC – peripheral blood mononuclear cells, ieAML- Immune experienced, wt-Wild type

IFN-G-Related Pathways in Breast Tumor Adjacent Tissues

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Objective

Breast cancers do not significantly benefit from current immunotherapy approaches which might be due to harsh immune suppressive conditions within the tumor microenvironment. The activity of interferon-gamma (IFN-g)-related pathways has been associated with the inflammatory conditions in various cancers that may hint the immunotherapy success. In this study, we aimed to assess the IFN-g signature in the histopathologically normal non-tumor breast tissues (n=165) which may give information on the inflammatory status in the periphery of the malignant area.

Materials and Methods

Tissues from reduction mammoplasty operations and hyperplasia were used as controls. RNA was isolated from non-tumor breast tissue at a distance of ~10 cm from the primary tumor tissue and converted into cDNA for qRT-PCR analyzes. The molecular changes of the 39 key genes of IFN-gamma pathway in non-tumor breast tissue were determined depending on the characteristics of the tumors, hormone receptor status, clinical stage and menopause stage of patients.

Results and Discussion

We observed that overall IFN-g pathway was regulated negatively in post-menopause stage. While negative regulator mTOR, PIAS4, PIK3CA, PIK3R1 genes and positive regulator RAPGEF1, CRKL, MAP3K11, CAMK2G MAP3K1 genes were downregulated, positive regulator JAK1, CAMK2D CASP1, IRF9 genes were up-regulated significantly. In clinical stage one and three, IFN-g pathways were upregulated but stage two showed down-regulation of positive regulator genes: PRKCD, AKT1, PTGES2, DAPK1, SMAD7, MAPK3, RAPGEF1, MAPK3K11 significantly. IFN-g pathway analysis in hormone receptor groups showed that IFN-g pathway was downregulated in HR+HER2- group with CAMK2G, DAPK1, CRKL, IFNGR1, IFNG, RAPGEF1 positive regulator genes. IFN-g pathway was upregulated in other receptor status. Positive regulator CAMK2A and PTGES2 genes were downregulated differently in HR+HER+ group. The HR-HER2- and HR-HER2- groups showed similar expression patterns, but unlike the other groups, the negative regulators SOCS1, PTNP11 and the positive regulator CEBPB genes were differentially down-regulated.

Keywords: breast cancer, Ifn-g, tumor microenvironment

The Effect Autophagy-Associated Secretome On Natural Killer Cell Activity

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Aim: Autophagy achieves a tumor-suppressing function in the initial stages of cancer by eliminating protein aggregates and damaged organelles that might promote genomic instability and lead to tumor formation. Autophagy induced by hypoxia or metabolic stress also plays an important role in the regulation of inflammatory pathways. In cancer cells, this may lead to secretion of pro- and/ or anti-inflammatory cytokines and chemokines which may help the immune escape of the tumor. However, the underlying mechanism of autophagy-mediated modulation of anti-tumor immune response is not yet fully understood. Our aim is to investigate whether chemotherapy-induced autophagy-associated secretome has the potential to modulate the NK cell-mediated anti-tumor immune responses.

Method: Initially, western blot and immunostaining analyses were performed for autophagy markers (LC3I/II and p62) to demonstrate that Etoposide (Eto) induces autophagy in MCF-7 cells. LC/MS-MS analysis was performed to determine the content of chemotherapy-induced autophagic secretome in supernatants of autophagy-induced MCF-7 cell cultures. Finally, how the chemotherapy-induced autophagic secretome effects the capacity of NK-92 cells to target MCF-7 cells was determined by degranulation assays.

Results: We demonstrated Etoposide (Eto) induces autophagy in MCF-7 cells as confirmed by detection of autophagy markers including LC3I/II and p62 by WB and by immunostaining analysis. LC/MS-MS results revealed that, metabolic enzymes, tumor antigens, chaperones and metastasis-related proteins were secreted during etoposide induced autophagy which could be reduced by use of Chloroquin. When wildtype or DNAM1 overexpressing NK-92 cells were treated with autophagic secretomes, it was observed that there were differences in the capacity of targeting MCF-7 cells.

Conclusion: This study provides new insights in the field of chemo-immunotherapy by characterizing the chemotherapy-induced autophagic secretome and determining its possible effect on NK cells.

Keywords: Autophagy, NK cell, cancer immunotherapy

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Keywords: Autophagy, NK cell, cancer immunotherapy

[PP-27]

Among Individuals Carrying Either HLA DQ B1 *02:01 Allele, The Development Of Caga Positive Helicobacter Pylori İnfection İs Significantly Higher

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Objective

CagA oncoprotein-containing strain of Helicobacter pylori is accused of causing gastric infection which also causes mucosal metaplasia. DQ cell surface protein plays an important role in the elimination of infectious agents. The aim of this study is to understand if there is a link between genetic polymorphisms of HLA DQB1 and H. pylori CagA IgA positive patients.

Materials and Methods

Patients with gastritis (n=174) were enrolled. CagA IgA, serum which shows active infection was analyzed by ELISA. HLA DQB1 allele frequency was analyzed by using PCR-SSO Method.

Results

According to our results, 134 patients are CagA Ig A negative while 40 patients are positive. There is no significant difference between gender and age distribution among 2 groups. HLA DQ B1 *02:01 allele frequency is 0,092 (32/348) among all patients. However, 17,5 % of CagA IgA positive group carry *02:01 allele (n=14/80) while only 6,7 % of CagA Ig A negative group carry DQB1*02:01 allele (n=18/268). Patients who carry HLA DQB1 *02:01 allele secreted CagA IgA immunoglobulin significantly higher frequency compared to non-*02:01 carriers (OR 0,33 (0,16-0,71) Maentel Haenzel Chi Square=8,56; p=0,003, 2-tailed).

Conclusion

HLA DQ B1 *02:01 allele carrying DQ protein on antigen-presenting cells might be less effective on surveillance and clearance of H. pylori with CagA oncoprotein which leads to the higher rate of infection.

Keywords: HLA DQB1 *02:01, Helicobacter pylori, CagA IgA

Coexpression Of Immune Checkpoint Receptors: Do We Need To Reconsider Immunotherapy Of Ovarian Cancer?

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Objective: Ovarian cancer has the highest mortality rate among all gynecological cancers. Due to its limited response to conventional chemotherapy, immunotherapy is a promising target. The aim of this study was to investigate the expression of immune checkpoint receptors (ICRs) on tumor infiltrating lymphocyte (TIL) and peripheral blood T cell subsets in patients with advanced ovarian cancer.

Materials and methods: Advanced stage ovarian cancer patients with stage III/IV (n=10) and healthy subjects (n=10) were included in the study. TILs were isolated from tumor tissue by using tumor dissociation kit. Expression of ICRs including PD-1, CTLA-4, LAG-3, Tim-3 and TIGIT on helper (Th) and cytotoxic T (Tc) cells were analyzed by flow cytometry. Th cell subsets were also analyzed by using subset-specific surface markers. Soluble plasma levels of ICRs were determined by ELISA.

Results: The ratios of Th1 and regulatory T (Treg) cells were significantly reduced but Th2 cells increased in tumor tissue compared to blood. Expressions of PD-1, CTLA-4 and Tim-3 on peripheral blood CD4⁺ Th and CD8⁺ Tc cells were significantly higher in cancer patients compared to healthy subjects. Tumor infiltrating Tc and Th cells had significantly increased expression of PD-1 and LAG-3 compared to blood. Coexpression of TIGIT with CTLA-4 and PD-1 was highest in Th and Tc cells infiltrating the tumor tissue compared to other samples. There was no difference in the plasma levels of soluble ICRs between study groups. Conclusion. Increase in Th2 cells with partially impaired Th1 and Treg responses in tumor microenvironment may be one of the main mechanisms mediating tumorigenesis. Higher expression of CTLA-4, PD-1 and Tim-3 in Tc and Th lymphocytes of patients might serve as targets for combined immunotherapy of ovarian cancer. Coexpression of ICRs is an important concept and may be the reason why some ovarian cancer patients fail to respond to anti PD-1/PDL1 immunotherapy.

Keywords: Cancer immunology, checkpoint inhibition, microenvironment, cytotoxic T cells, T helper cells

Evaluation of SARS-CoV-2 Specific Antibody Responses in Asymptomatic and Symptomatic Cases

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Purpose: SARS-CoV-2 emerged in China at the end of 2019 and developed rapidly, causing a global epidemic worldwide. Clinical syndrome caused by SARS-CoV-2 is highly variable. It has been shown that many people can carry the virus without showing any symptoms. In this study, virus-specific antibody response in the SARS-CoV-2 global epidemic was evaluated. In addition, it has been investigated that protective immune response may develop in asymptomatic individuals during the epidemic.

Materials-Methods: The study was included 57 SARS-CoV-2-infected (7 of the 57 cases are asymptomatic) and 70 uninfected cases. The levels of IgG-Spike-Protein (IgG-SP) and IgA-Spike-Protein (IgA-SP) antibodies were evaluated. None of the individuals had received a specific COVID-19 vaccine at the start of the study. Serum samples were obtained from infected cases at the beginning, at the 1st, 3rd and 6th months of follow-up. In addition, IgG-Spike-antibody responses of the cases after the 2nd dose of CoronaVac vaccine (28th day) were also evaluated.

Results: In both symptomatic (n=50) and asymptomatic individuals (n=7), IgG-SP-levels decreased compared to baseline levels at 6th months, while IgA-SP-levels were increased significantly. IgA-SP-levels were significantly increased at baseline in symptomatic cases compared with asymptomatic cases. In symptomatic and asymptomatic cases, IgG-SP-levels increased after vaccination compared to pre-vaccination. Among the vaccinated subjects, those who were infected had IgG-SP-levels approximately twice that of the uninfected cases.

Conclusion: SARS-CoV-2 specific antibody levels were found to be higher at baseline in symptomatic cases. However, in all infected cases, significantly reduced IgG-SP-levels were detected at 6th months. With vaccination, IgG-SP-levels were shown to increase effectively. Our data support that it is possible to detect virus-specific antibody positivity in asymptomatic individuals, and this is an indicator of immune response. Antibody screening has the potential to determine herd immunity, therefore it is clearly stated that detecting antibody levels is crucial.

Keywords: SARS-CoV-2, humoral immunity, IgG-Spike, COVID-19

The Effect Of Age And Gender Differences On The Distribution Of T And B Lymphocyte Subsets

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Objective: Aging and gender differences are known to be variables affecting the functions of the immune system. It is known that there are differences in the distribution of T and B lymphocyte subsets, especially from birth to post-puberty. This study analyzed the effect of age and gender differences on the distribution of T and B lymphocyte subsets in healthy adults. **Materials-Methods:** Peripheral blood samples were obtained from 118 healthy adult individuals (60 males and 58 females) aged between 20 and 60 years. T and B lymphocyte subsets were analyzed by flow cytometry according to whole blood lysing protocol.

Results: The healthy individuals were classified according to both gender and age: 20-29, 30-39, 40-49 and 50-60. No significant difference was found in B lymphocyte subsets and CD8⁺ T according to gender and age groups. CD3⁺ and CD4⁺ T lymphocyte ratios were higher in females than males. Age and gender differences have not affected the ratio of naive, central memory, effector memory and effector memory-CD45RA T (TEMRA) in CD4⁺ T lymphocytes but decreased naive and increased TEMRA subsets in CD8⁺ T lymphocytes were found in the 50-60 age group. Regulator T (Treg) cells were increased in individuals aged 50-60 years than in aged 20-49. Follicular helper T (TFH) cells were decreased in females than in males. Elevated Th2 and Th17 cells in females aged 20-29, and elevated Th1 cells in females aged 30-60 years were obtained.

Conclusion: Our results showed that age differences did not affect, CD3⁺, CD4⁺ and CD8⁺ T lymphocyte ratios in adults, but gender differences affect CD3⁺ and CD4⁺ T lymphocyte ratios. It also showed that age and gender differences did not affect B lymphocyte subsets. It is thought that these findings might be used as a reference in routine analyzes.

Keywords: B lymphocyte, Flow cytometer, Lymphocyte subsets, T lymphocyte

Human Memory T Cell Dynamics After Aluminum-Adjuvanted Inactivated Whole-Virion SARS-CoV-2 Vaccination

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Objective: The magnitude and dynamics of memory T cell responses determine the efficacy and durability of protection conferred by a vaccine. This study evaluates the functional capacity of CD4⁺ and CD8⁺ terminally-differentiated effector (TEMRA), central memory (TCM), and effector memory (TEM) cells obtained from the volunteers vaccinated with an aluminum-adjuvanted inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac) in a phase III clinical trial.

Materials-Methods: Peripheral blood samples were collected from the placebo group and the individuals vaccinated with the inactivated SARS-CoV-2 vaccine (CoronaVac, Sinovac Life Sciences Beijing, China) on the days 7 and 120 following the second dose. Seven days and four months after the second dose of the vaccine, the memory T cell subsets were collected and stimulated by autologous monocyte-derived dendritic cells (mDCs) loaded with the SARS-CoV-2 spike glycoprotein S1.

Results and Conclusions: Compared to the placebo group, memory T cells from the vaccinated individuals significantly proliferated in response to S1-loaded mDCs. Proliferation kinetics of TEMRA, TCM and TEM subsets were compatible. CD4⁺ and CD8⁺ memory T cell proliferation was detected in 86% and 78% of the vaccinated individuals, respectively. More than 73% and 62% of the vaccinated individuals harbored TCM and/or TEM cells that responded to S1-loaded mDCs by secreting IFN- γ . The expression of CD25, CD38, 4-1BB, PD-1, and CD107a indicated the modulation of memory T cell responses in CD4⁺ and CD8⁺ memory T cells. Especially on day 120, PD-1 was upregulated on CD4⁺ TEMRA and TCM, and on CD8⁺ TEM and TCM cells; accordingly, proliferation and IFN- γ secretion capacities tended to decline after 4 months. the combination of inactivated whole-virion particles with aluminum adjuvants possesses capacities to induce functional T cell responses and support the establishment of T cell memory.

This study was supported by Health Institutes of Turkey (Project no: 2020cv01-9342/9401).

Keywords: COVID-19, T cell, dendritic cell, Interferon-gamma, proliferation

Oil Base Emulsions: Used as Vaccine Vehicles for Water-in-Oil Emulsion

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Vaccine adjuvants are chemicals, microbial compounds, natural products, etc., that increase the immune response to vaccine antigens. Interest in reducing vaccine-related side effects and inducing specific types of immunity has led to the emergence of many new adjuvant formulas. The purpose of vaccination is to generate immune responses against the administered antigen that should provide long-term protection against infection. One of the most important factors increasing the immunogenicity of these vaccines is the adjuvant. There are numerous adjuvant types used in veterinary vaccinations, but the most popular ones for inactivated viral vaccines include alum and mineral oil-based adjuvants with or without saponin. The short-lived antibody responses induced by vaccines with alum and saponin as adjuvants necessitate relatively frequent revaccinations at intervals of 6 or even 4 months. Oil-based adjuvant vaccines, in contrast, seem to offer several benefits, including the generation of large titers and long-lasting antibody responses, leading to more effective protection. Different types of emulsions have been developed for this purpose. In vitro antigen release and in vivo immunogenicity are associated with O/W emulsions with the fastest in vitro release and lowest immunogenicity, followed by W/O/W and slowest antigen release and W/O with highest immunogenicity. Although mineral oil-based W/O emulsions are known to provide powerful and long-lasting immunity, they occasionally can react with reactive antigens. Antigens are kept in emulsions and gently oscillate over a lengthy period. W/O emulsions help to reduce the vaccine dose or antigen concentration, which is important because vaccines should be cost-effective, as they create a strong immune response. W/O formulations can also enhance the cellular immune response. Various screening studies in mice with viral, bacterial, or parasitic antigens have shown that W/O emulsions induce higher IgG2a antibody levels than other emulsion types.

Keywords: Adjuvant, Oil-based adjuvant, Vaccine, Water-in-Oil Emulsion

[PP-33]

CD66b⁺ Monocytic Cells in the Tumor Microenvironment

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The monocyte subsets are commonly distinguished according to CD14 and CD16 surface markers. Recently our lab group discovered an unconventional subset of monocytes significantly increased in the circulation of cancer patients which was determined with CD14⁺CD15⁻CD33⁺HLA-DR⁺CD66b⁺ immunophenotype. These CD66b-positive monocytes were characterized with increased phagocytosis, migration and adhesion capacities when compared to other monocyte subpopulations, and apart from these abilities, they stimulate high levels of IFN-gamma secretion and T cell proliferation. High numbers of CD66b⁺ tend to correlate with good prognosis. In the current study, the percentage distribution of cells in breast and colorectal cancer patients' tumor tissues were tested together with additional myeloid surface markers such as CD45, CD14, CD16, CD66b, CD206, CD163, HLA-DR, CCRL2, CD80, CD86 by multicolor flow cytometry. Peripheral blood was used as a control. The percentage of the CD66b⁺ monocyte in tumor for both breast and colon cancer was higher than the peripheral blood and non-tumor tissue. These tumor-infiltrating CD45⁺HLA-DR⁺CD14⁺CD66b⁺ cells were characterized to be CD163⁻CD206⁻ which confirmed the non-macrophage phenotype of this sub-population. CCRL2, CD80 and CD86 expression was also of indicative for their pro-inflammatory assets. In conclusion, CD66b-positive monocytes are also present in the tumor microenvironment and retain their monocytic phenotype.

Keywords: Tumor microenvironment, monocytes, myeloid cell differentiation, monocyte subtypes

Poorly Regulated Humoral Immune Responses In MIS-C

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Aim

MIS-C (multisystem inflammatory syndrome in children) is a clinical condition that may be manifested by symptoms such as fever, abdominal pain, headache, vomiting, and diarrhea that may occur in children with SARS-CoV-2 infection. B regulatory cells (Bregs) are immunosuppressive cells that support immune tolerance and suppress pathological immune responses. B cell exhaustion is associated with weaker antibody responses to pathogens. This study aimed to investigate the role of B cell subsets in the pathogenesis of MIS-C.

Methods

17 MIS-C cases, 17 pediatric COVID-19 and 17 healthy controls were included in the study. flow cytometric evaluation was performed using a 10-color mAb panel from peripheral blood samples.

Results

-CD19+ B cells were significantly increased in MIS-C compared with COVID -19 and the healthy control group. Consistent with this increase, IgM and IgD expression were found to be increased in MIS-C patients compared to COVID -19 patients and healthy controls. - Naive B cells (CD27-IgD+) were found to be increased in MIS -C patients compared with COVID-19. Switched memory and IgM memory cells (CD27+IgD- and CD27+ IgD+) also showed a similar profile.

A significant decrease in MIS-C cases was observed in immature/transient B cells (CD24highCD38high) and primary memory B primarily cells (CD24highCD38-) compared to COVID-19 and healthy controls. Regulatory B10 cells (CD24highCD27-) was decreased in COVID-19 and MIS-C compared to the healthy control group.

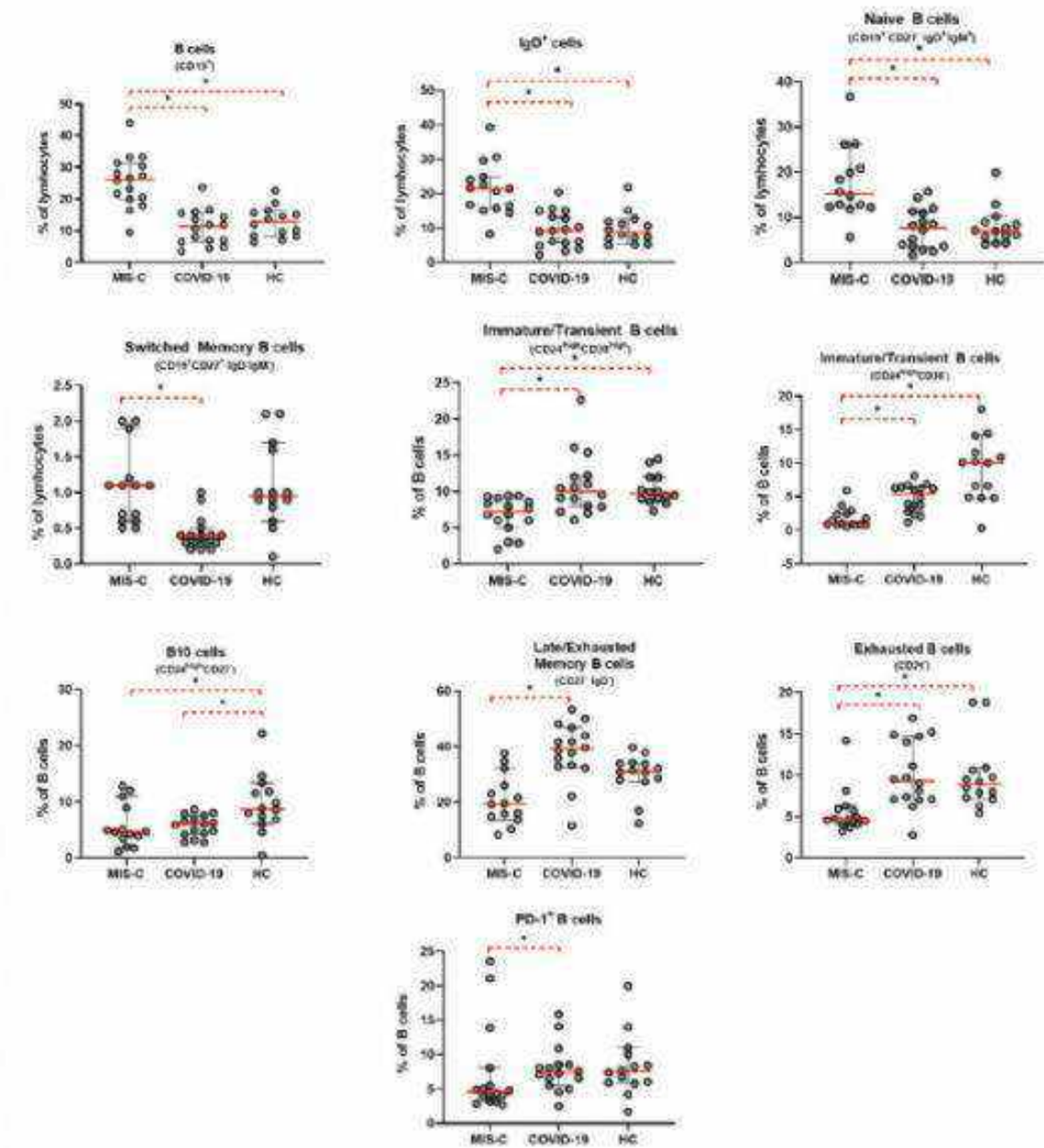
-Late/exhausted memory cells (CD27-IgD-IgM-) were decreased in MIS-C compared to COVID-19. CD21-exhausted B cells and PD-1+ B cells followed similar course.

Conclusions

According to the data obtained, the B-cell profile in MIS-C cases differs from COVID-19 in favor of inflammation. The effect of inflammation-limiting regulator B cells seems to be lower in MIS-C than in COVID-19, suggesting the presence of an aggressive and poorly regulated humoral immune response in MIS-C.

Keywords: MIS-C, COVID-19, B cell, Breg, exhaustion

B cell subsets were significantly different between COVID-19 and MIS-C



Relationship Interferon Signalization and Disease Activation in Patients with Juvenile Scleroderma

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INTRODUCTION

Juvenile dermatomyositis (JDM) is an inflammatory myopathy characterized by skin involvement, vasculitis, and progressive muscle weakness. Increased interferon signaling is thought to play a role in the pathogenesis of JDM and is associated with disease severity and activation, as well as the development of calcinosis. Juvenile scleroderma (JSc) is a heterogeneous group of diseases associated with sclerotic skin lesions, grouped together as systemic sclerosis and localized scleroderma (morphea). Although JSc has a different skin involvement than JDM, some similarities in their pathogenesis can be identified. The aim of this study is to measure the levels of cytokines and chemokines involved in interferon signaling in patients with JDM and JSc and to determine their correlation with disease severity.

METHODS

Thirty-one JSc, 5 JDM, and 13 healthy controls were included in the study. Patients with morphea were scored according to the LoSCAT (activity index) and LoSDI (damage index) indices. Cytokines and chemokines involved in interferon gene signaling (IL-8, IL-1, IP-10, MCP1, TNF- α , CXCL-10, IFN- α , IFN- β , IFN- γ) were measured by ELISA.

RESULTS

The ratio of female to male patients was 24/12, the median age was 14 years (min 4, max 18), and the median follow-up time was 36 months (min 12, max 108).

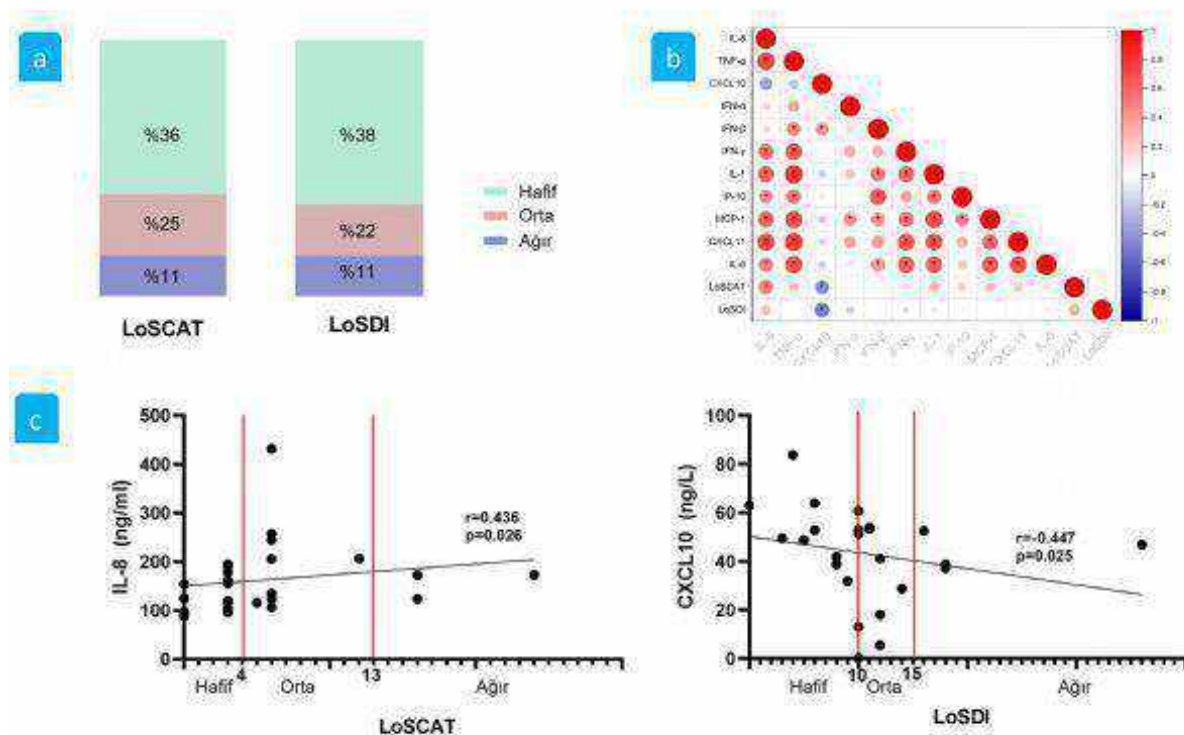
It was found that IL-8, IFN- β , IP-10, and MCP-1 were significantly decreased in patients with JSc compared with the healthy control group. TNF- α and IFN- γ were found to tend to decrease in patients with JSc, whereas IFN- α tended to increase in patients with JDM. The correlation of LoSCAT and LoSDI indices with cytokine and chemokine levels in patients with JSc is summarized in Figure 1.

CONCLUSION

The results suggest that interferon signaling may be impaired in patients with JDM and JSc, and that cytokines whose significant changes were observed in this study may play a key role in monitoring disease activity.

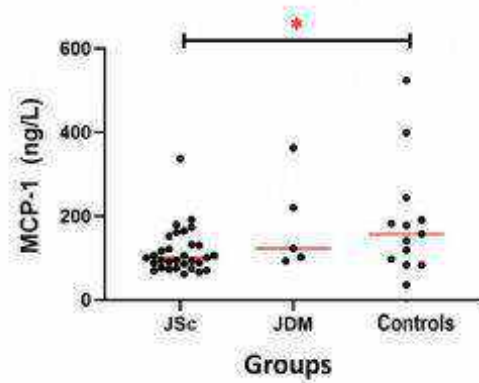
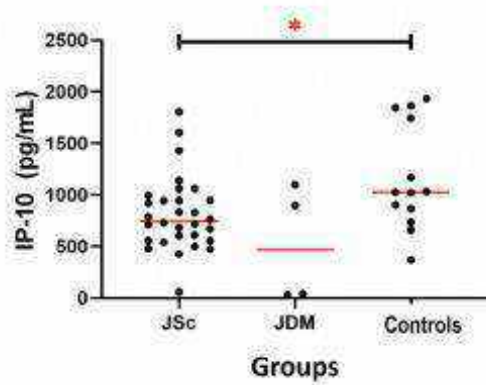
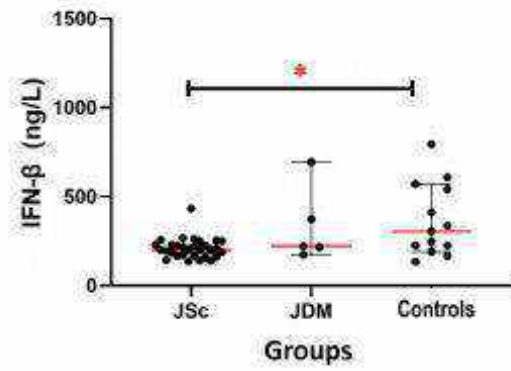
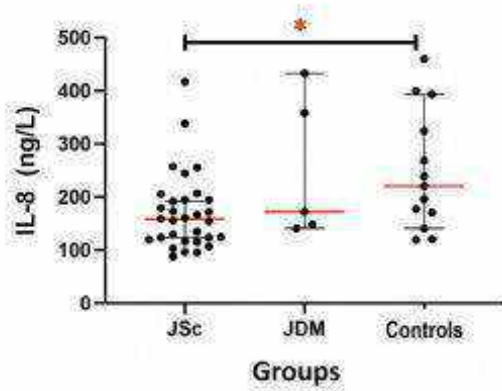
Keywords: morphea, juvenile scleroderma, interferon, LoSCAT, LoSDI

Correlation of LoSCAT/LoSDI Assessments of Patients with Localized Scleroderma with Cytokines and Chemokines



a. LoSCAT/LoSDI-Morphea frequency, b. Cytokine and chemokine correlation map, c. Statistically significant correlations

Cytokine and chemokine distribution in patient and healthy control groups



Altered Immune Cell-Derived Exosomes in COVID-19

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Aim

COVID-19 is a disease characterized by acute respiratory failure that causes a major global healthcare issue. Exosomes are small lipid vesicles derived from multivesicular bodies and released from many cell types. Exosomal cellular cargo reflects the type of cell from which it originates. With this study, we aimed to contribute to the literature on the possible role of exosomes in the immunopathogenesis of COVID-19.

Method

Our study included patients diagnosed with COVID-19 and classified according to their pneumonia status. Six patients each from the uncomplicated, mild, and severe pneumonia disease groups and 10 healthy controls were included in the study. Exosomes from serum samples were extracted using a commercial isolation kit. In the pre-designed panel, after staining with monoclonal antibodies (mAbs), the exosomes were immunophenotyped using Flow Cytometry.

Results

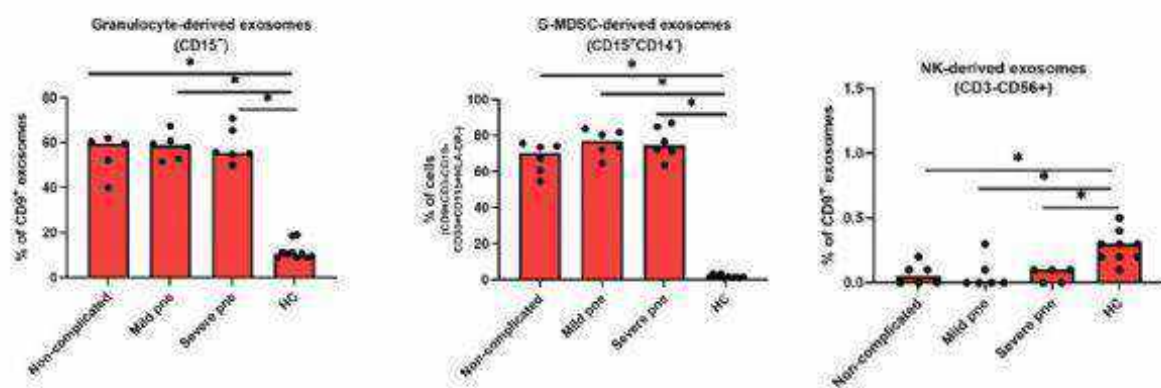
The presence of exosomes was confirmed by staining with exosome-specific mAbs CD9, CD63, and CD81. According to our results, exosomes derived from granulocytes and G-MDSC (granulocyte-like myeloid suppressor cells) (%) were significantly higher in COVID-19 patients than in healthy groups. Consistent with the lymphocytopenia observed in COVID -19, lymphocyte-derived exosomes were markedly reduced in the disease stage. In addition, it was observed that NK-derived exosomes were significantly decreased even at the mildest disease severity.

Conclusions

Taken together, we found that NK cell, granulocyte and G-MDSC-derived exosomes may play a role in COVID-19. Although there is no significant variation of exosomes with disease severity, the significant increase found even in uncomplicated cases compared with healthy controls draws attention to the possible role of exosomes in COVID-19.

Keywords: COVID-19, SARS-CoV-2, exosome, extracellular vesicles, G-MDSC

Significantly altered exosomes in COVID-19



Regulation Of NLRP3 Inflammasome-Related Gene Expressions in The Progression To Chronicity In Brucellosis With Bone Joint Involvement

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Aim

Bone joint involvement (BJI) is commonly observed in the zoonotic infection, brucellosis, caused by the intracellular pathogen *Brucella*. Caspase-1 and caspase-11 initiate joint inflammation and proinflammatory cytokine production and participate in infection control thereafter. Caspase-1 and Caspase-11 involve inflammation by activating the NLR family pyrin domain containing 3 (NLRP3). The role of NLRP3 dysfunctions in bone and joint diseases was shown, however, the function of NLRP3 in the chronicity of BJI+ Brucellosis remains unknown. Our study determined the mRNA levels of genes involved in NLRP3 inflammasome activation during the chronicity process in BJI+ brucellosis.

Method

The study included ten acute and six chronic BJI+ brucellosis cases and eight healthy controls. Cases were classified as acute (0-2 months) and chronic (>12 months) according to the onset time of symptoms. A human mRNA microarray analyzed the expression of over 30,000 genes in mononuclear cells separated from peripheral blood by Ficoll density-gradient centrifugation. An independent sample T-test analyzed the changes of genes in BJI+ brucellosis. A KEGG pathway analysis determined the association of those genes with NLRP3 expression and activation.

Results

NLRP3 mRNA was decreased in BJI+ chronic brucellosis compared to control ($p=0.015$). Supporting this, TRIM31, which is involved in the degradation of the NLRP3 inflammasome, increased in these cases compared to the control ($p=0.009$).

In contrast; P2RX7, which plays a role in forming the NLRP3 complex by uptake of extracellular ATP into the cell, was increased in BJI+ acute brucellosis compared to the control ($p=0.015$). Similarly, NEK7, which acts as a component of the NLRP3 inflammasome complex, increased in these cases ($p=0.019$ and $p=0.013$).

Conclusion

Our findings suggest that the expression NLRP3-related genes increase in BJI+ acute brucellosis, whereas these pathways are suppressed at the mRNA level in BJI+ chronic brucellosis.

Keywords: Brucellosis, mRNA, inflammasome, NLRP3

[PP-39]

The Role Of Cytotoxic T And Treg Cell Subsets And Exhausted T Cells In The Pathogenesis Of MIS-C

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Aim

Multisystem inflammatory syndrome (MIS-C) manifests as fever, inflammation, and multiple organ damage in children after infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). While the disease is usually mild in children infected with SARS-CoV-2, the reason why some patients develop mis-c remains unclear. Studies at COVID-19 continue to show the importance of T cells. In our study, we aimed to uncover the potential role of cytotoxic T cell subsets (CTL) and regulatory T cells (Treg) in MIS-C.

Methods

17 MIS-C, 17 pediatric COVID-19 cases and 17 healthy controls were included in the study. Flow Cytometry evaluation was performed with a 10-color MoAb panel from peripheral blood samples.

Results

It was observed that effector memory T cells (EM; CD3+CD8+CD45RA-CCR7-) were decreased in MIS-C compared with pediatric COVID-19 cases and healthy control group. Effector memory 1 (EM1; CD45RA-CCR7-CD27+CD28+) CD8+ T cells increased in MIS-C compared to pediatric COVID-19 cases. Effector memory 2 (EM2; CD45RA-CCR7-CD27+CD28-) CD8+ T cells decreased in MIS-C compared with pediatric COVID-19 cases and healthy controls. Exhausted CD8+ T cells (PD-1+), naive (CD45RA+FoxP3low), activated effector (CD45RA-FoxP3high), and nonsuppressive (CD45RA-FoxP3low) Treg cells, decreased in MIS-C cases compared with pediatric COVID-19 cases and healthy controls.

Conclusions

Decreased frequency of CD45RA-CCR7- EM CD8+ T cells in peripheral blood that have the ability to migrate to non-lymphoid tissues, increased in rapidly activated CD8+ T cells (EM1, EM2) expressing the costimulatory molecule CD28, increased E CD8+ T cells, re-expression of CD45RA in antigen-induced senescent T cells, and down-regulation of CD28 and CD27 expression, as well as decreased abundance of exhausted CD8+ T cells and Treg cells, may be associated with persistent excessive inflammation and multiple organ damage in MIS -C patients.

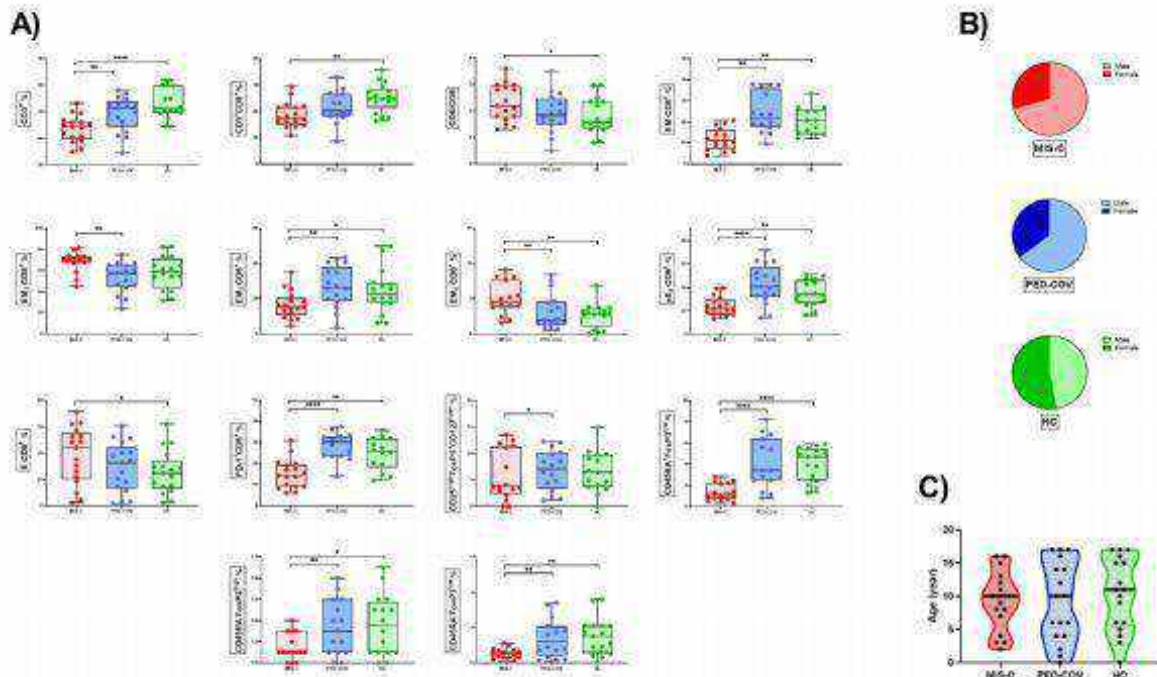
Keywords: MIS-C, COVID-19, CD8+ Cytotoxic T cell, Treg, exhaustion

A)

B)

C)

The frequency of cytotoxic T-cell and Treg-cell subsets determined by flow cytometry analysis in MIS -C and pediatric COVID -19 patients and healthy control subjects is shown (A). The gender distributions of the groups are shown (B). The age distributions of the groups are shown (C). The black lines represent the median values for each group. In all analyses, $p < 0.05$ was considered to indicate statistical significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). Abbreviations: MIS-C: pediatric patients with multisystemic inflammatory syndrome; PED-COV: pediatric COVID-19 patients; HC: healthy controls.



The frequency of cytotoxic T-cell and Treg-cell subsets determined by flow cytometry analysis in MIS -C and pediatric COVID -19 patients and healthy control subjects is shown (A). The gender distributions of the groups are shown (B). The age distributions of the groups are shown (C). The black lines represent the median values for each group. In all analyses, $p < 0.05$ was considered to indicate statistical significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). Abbreviations: MIS-C: pediatric patients with multisystemic inflammatory syndrome; PED-COV: pediatric COVID-19 patients; HC: healthy controls.

[PP-40]

Early Activation in Memory And Naïve Th Cells Are Distinctively Monitored By CD69 And CD154 Expression

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In this study, costimulatory activity of myeloid cells with different maturation states were used to stimulate to naïve (T_n), central memory (T_{cm}) and effector memory (T_{em}) CD4⁺ T cell sub-populations. Distinctions in the entry into the early activation phase were assessed by CD69 and CD154 upregulation kinetics by flow cytometry at different time points. CD14⁺ monocytes were isolated by magnetic-associated cell sorting (MACS) from peripheral blood mononuclear cells (PBMCs), immature dendritic cells (imDC) and mature dendritic cells (mDC) were generated. CD4⁺ T_n, T_{cm} and T_{em} cells were purified by FACS according to CD45RA, CD45RO and CCR7 expression. KG-1a, U937, HL-60, Kasumi-1, THP-1, monocytes, imDC and mDC cells were co-cultured with T cell subpopulations. CD69 and CD154 expression were assessed by flow cytometry at 3, 6, 12, 24 and 48h of co-culturing under anti-CD3 stimulation. Memory CD4⁺ T cells entered early activation state more slowly but also exit from this state more quickly than naïve CD4⁺ T cells. As the maturation state of the myeloid cells increased, they induced higher CD69 expression for 24h. However, in contrast to more immature cells, mature myeloid cells did not highly induced CD154 expression. Especially in T memory cells, CD69 and especially CD154 expression was not as drastic as in T_n. Therefore, memory T cells' entry into the early activation stage may have distinct kinetics than naïve T cells.

Keywords: Helper T cells, Myeloid cells, Co-stimulation

Investigation of The Effect Of Oleuropein On Mononuclear Inflammatory Cell Responses in the Sjögren Syndrome Murine Model

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INTRODUCTION-Aim: In this study, it was aimed to investigate the effect of Oleuropein on mononuclear inflammatory cell responses and symptoms in Sjögren's Syndrome (SS) murine model. It is aimed to develop a treatment option that supports the current treatment in SS, which is an autoimmune inflammatory disease, and to contribute to the literature on supportive treatments.

Methods: Our study was conducted in an SS murine model in 8-10 week old male Balb/c mice. To evaluate the effect of oleuropein, the SS model was created with Ro-60-273-289 peptide immunization. By oral administration 15mg/kg, 30mg/kg, 45mg/kg of oleuropein was given for 28 days. In splenocytes after sacrifice CD4+T lymphocytes which secrete IFN- γ , IL-17, IL-10 were analyzed. Lymphocytic infiltrate in glandular tissues was analyzed by H&E staining. Saliva rate was measured by collecting saliva samples for 10 minutes. **RESULT-Conclusion:** Due to increasing doses of oleuropein, CD4+IFN γ + and CD4+IL17+ cells were significantly decreased ($p<0.05$) in the treatment groups compared to the SS group. Lymphocytic infiltrate was significantly reduced (focus score <1) ($p<0.05$) at Oleuropein doses of 30mg/kg and 45mg/kg compared to the SS group. Saliva rate increased with the increasing doses of Oleuropein.

Keywords: Sjögren syndrome, autoimmune disease, oleuropein, murine model

Follicular Cytotoxic T Cell Subsets And Their Functions In Healthy Individuals

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OBJECTIVES:

A subset of CD8⁺ T cells expressing CXCR5, called follicular cytotoxic T (TFC) cells are localized to B cell follicles in lymph nodes, and eliminate infected or malignant B and TFH. Recent studies showed that CD40L and ICOS expression on TFC might be associated antibody responses in B cells. In this study, the functional differences of TFC subsets were investigated in healthy individuals.

Method:

Peripheral blood mononuclear cells (PBMC) were isolated from whole blood by ficoll (n=20). CD19⁻ cells were sorted and were stimulated by PMA/ionomycin for 5 hours, then were stained with anti-CD3, -CD8, -CXCR5, -CD40L and -ICOS. After fixation/permeabilization, intracellular staining was performed with anti-IL-4, -IL-17, -IL-21, -IFN- γ , perforin and granzyme-B. CD40L⁺CXCR5⁺CD8⁺, ICOS⁺CXCR5⁺CD8⁺ and CXCR5⁺CD4⁺ cells were sorted and co-cultured with CD19⁺ cells for 5 days with anti-CD3/CD28. Plasmablast ratio and viability of CD19⁺ cells were analyzed by flow cytometry (n=2).

Results:

TFC were divided ICOS⁺, CD40L⁺ and ICOS⁻CD40L⁻ (DN) TFC according to ICOS and CD40L expressions. The ratio of DN TFC were increased compared to ICOS⁺TFC and CD40L⁺ TFC. IL-21, IFN- γ , perforin and granzyme-B expression were increased in ICOS⁺ TFC than CD40L⁺ TFC and DN TFC. Overall, DN TFC had lower levels of cytokines, perforin and granzyme-B than ICOS⁺ TFC and CD40L⁺ TFC. The viability of B cells was decreased in ICOS⁺ TFC co-cultured, but plasmablast differentiation were increased in CD40L⁺TFC co-cultured.

Conclusions:

ICOS⁺ TFC had higher cytotoxic proteins, and reduced viability on B cells, suggesting that ICOS⁺ TFC might have a greater cytotoxic effect. On the other hand, CD40L⁺ TFC have a stimulating effect on B cells rather than a cytotoxic effect due to the low content of perforin and granzyme-B levels and cause plasmablast transformation in B cells.

This project was supported by I.U. BAP (project no: 38452) and TUBITAK (project no: 122S204).

Keywords: Cytotoxic CD8 T cells, Follicular cytotoxic T cells, B cells

The Immunomodulatory Effects Of Folate On Macrophage Responses To Aluminum Adjuvanted Vaccine

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Objective: Aluminum salts remain the most common adjuvants in licensed vaccines, whereas the impacts' on innate immunity are still elusive. Here, we investigated how the aluminum adjuvanted vaccine (AAV) affects macrophage biology under the influence of folate, and Toll-like receptor-4 mediated activation.

Materials-Methods: AAV was applied to J774.A1 cells in different concentrations with or without lipopolysaccharide stimulation and incubated in a high or low folate medium. Cells were examined for; folate receptor-2 (FOLR2), F4/80, CD206, CD11b, CD69, CD62L, MHC-II, CD40, CD80, CD86 marker expressions, phagocytosis analysis, reactive oxygen species (ROS) and nitric oxide (NO) production capacity by flow cytometry or immunofluorescence microscopy, aluminum uptake by lumogallion staining, and proinflammatory gene expressions by RT-qPCR analysis. We also investigate FOLR2 expressing macrophage infiltration in AAV injected skeletal muscle in mice by immunophenotyping CD11b, F4/80, and CD206 markers.

Results and Conclusions: As expected, AAV supported macrophage maturation and activation in general. Conversely, phagocytosis activity was reduced dramatically by the abundance of aluminum uptake. The higher folate content modulated the unstimulated macrophage responses positively. Specifically, MHC-II expression and phagocytosis activity were supported by folate content both in unstimulated and activated macrophages, whereas the positive impact on ROS and NO activity is attenuated or reversed by increasing AAV concentration and activation. On the other hand, most of the activated macrophage responses to AAV were partially inhibited by increased folate content. Next, we identified that in the AAV injected muscle, FOLR2 is expressed more specifically by CD206 positive macrophages. In summary, macrophage activation under the modulatory effects of folate can change the response to AAVs. According to these observations, we can propose that further research on folate may support vaccine efficiency and safety as an active vaccine component.

Keywords: Folate, vaccine, adjuvant, alum, macrophage

Evaluation Of Antinuclear Antibody (ANA) Test And Pattern Distribution In Patients In A University Hospital: A Cross-Sectional Study

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Purpose: The antinuclear antibody (ANA) test, despite all the downsides, is still considered and used as the gold standard technique amongst all the other options to diagnose autoimmune diseases. Our aim was to investigate the positivity rate and pattern distribution of ANA test results as well as the relationship between test results and certain demographic characteristics of patients.

Methods: In this cross-sectional study, the ANA test results of 3469 adult patients who were diagnosed and classified by rheumatologists, other clinicians were evaluated between January-May 2022 in Dokuz Eylul University Hospital Immunology Laboratory. All samples were studied with indirect immunofluorescence antibody (IIFA) assay on HEp-2 cells (HEp-2-IIFA) and staining patterns were evaluated. In the analysis of the data, descriptive statistics [mean (sd), median (interquartile range (IQR)), percent (%)], chi-square test and Mann-Whitney u test were used according to their suitability. Statistical significance was quantified at the $p < 0.05$ level in all tests.

Results: The median age of the patients included in the study was 52.00 (39.00-65.00) years and 65.9% (n=2286) of the research group consisted of women. When the anti-cell (AC) autoantibodies were evaluated according to The International Consensus on ANA Patterns; ANA positivity was detected in 59% (n=2048) of the patients, while AC-0 (negative) was found in 41.0% (n=1421) of the patients. "AC-0", "AC-4,5", "AC-2", "AC-8,9,10", "AC-21", "AC-19,20" patterns were the most frequent patterns observed. ANA positivity was significantly higher in females than males ($p < 0.001$). It was observed that the rate of ANA positivity in outpatients was significantly higher than in hospitalized patients ($p < 0.001$). ANA positivity rate was also found higher in patients older than 50 years of age ($p = 0.022$).

Conclusions: Nearly three-fifths of the patients studied were HEp-2-IIFA-positive. Individuals aged 50 and over and female patients had more positive results. As a result, female patients and elderly patients may need to be evaluated more carefully in terms of ANA positivity.

Keywords: antinuclear antibodies, anti-cell autoantibodies, ANA patterns, HEp-2 cells, indirect immunofluorescence

[PP-46]

Assess Various Cytokine Levels After Receiving Different Types Of Vaccines Against Covid-19 Infection

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Zarqa University

Purpose: This Work Was Designed To Assess Various Cytokine Levels After Receiving Different Types Of Vaccines Against Covid-19 Infection.

Method: One Hundred And Six aberrantly Healthy Donors Categorized Into 60 Cases Vaccinated With Pfizer And 25 With Sinopharm, 15 With Astrazeneca And 6 Non-Vaccinated Cases Were Enrolled In This Study. Serum Levels Of Interleukin (Il)-6, Il-10, Il-33, Interferon Gamma (Ifn- Γ) And Tumor Necrosis Factor Alpha(Tnf-A) Were Measured Using Enzyme-Linked Immunosorbent Assay (Elsa)

Results: Il-10 Is Significantly Decreased ($P < 0.05$) In Not Vaccinated Patients Compared To Vaccinated Ones. Insignificant Change Was Recorded In All Cytokines Between Vaccinated Patients With Different Types Of Vaccines. Covid-19 Patients Did Not Show A Significant Change In Levels Of Measured Cytokines.

Conclusion: Selected Cytokines Did Not Differ Regarding Type Of Covid-19 Vaccine. More Prospective Studies With Larger Sample Size Are Warranted To Validate Our Results.

Keywords: Vaccine, Cytokine, Covid-19

In vitro Modulating Effect of IL-2 on T Helper Cell Populations

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Autoimmune pathogenesis has been associated with high T follicular helper (Tfh) and T peripheral helper (Tph) cells and regulatory T (Treg) cell defect in autoantibody mediated autoimmune diseases. IL-2 is the major cytokine in helper T cell homeostasis and the main inducer of the Treg cell population. This study aims to examine the effect of IL-2 on helper T cells in the healthy donors.

Ten healthy donors (women/men: 5/5, median age (range): 46 (29-64) years) were included in the study. Peripheral blood cells of the donors were cultured with RPMI 1640 medium containing 10% fetal bovine serum for 1 or 5 days in several concentrations of IL-2 (0, 10, 30, 100 IU/ml). After the culture period, cells were evaluated by surface- and intracellular-staining (FOXP3) with antibodies at flow cytometry. CD4+CD25highCD127- or CD4+CD25+FOXP3+ cells as Treg cells and CXCR5, ICOS and PD-1 expression on CD4+ T cells for Tfh cells were analyzed. Wilcoxon matched pairs signed rank test was used for statistical analysis. At the optimization experiments, 10 IU/ml IL-2 and 5 days of stimulation period has been chosen for the optimal effect detection. CD4+CD25high (p =0.015), CD4+CD25highCD127- (p < 0.001), CD4+FOXP3+ (p = 0.002) and CD4+FOXP3+CD25+ (p < 0.001) T cell populations associated with Treg cells were increased with IL-2 stimulation. IL-2 decreased CD4+CXCR5+ICOS+ (p = 0.002) T cell population associated with Tfh cells. A decreasing effect of IL-2 was also observed on Tph-related CD4+CXCR5-PD-1+ (p = 0.048) and CD4+CXCR5+ICOS+ (p = 0.003) phenotypes of T cells.

IL-2 has a modulating effect on Treg and ICOS or PD-1 expressing helper T cells. This effect of IL-2 tends to be on ICOS rather than PD-1 expression of T helper cells. Low-dose IL-2 treatment may provide a new option for autoimmune conditions such as myasthenia gravis, which will be pursued with these findings.

Keywords: IL-2, Tfh, Tph, Treg, autoimmune diseases

SARS-Cov-2 Spike Specific Igg Persists For 6 Months Post-COVID-19 in Convalescent Pediatric Patients

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Objective: SARS-CoV-2 virus causes the disease COVID-19 which can cause various outcomes across different ages. Understanding the immune responses of children to the virus is crucial since pediatric patients demonstrate milder or no symptoms while adults are affected more. In this study, SARS-CoV-2 spike specific IgG, IgG1 and IgG4, responses in convalescent pediatric patients were investigated throughout different timepoints; at 1st, 3rd and 6th months after their last positive PCR and results were analyzed between different ages and disease severities.

Materials-Methods: Convalescent pediatric patients between the ages of 18 months-18 years old were selected after 25 days have passed since their last positive PCR test. Children in the same age group who had not been infected were selected as healthy controls. Blood plasma were collected and SARS-CoV-2 specific IgG, IgG1 and IgG4 responses were detected with indirect ELISA, developed and optimized in our laboratory. Results were analyzed using GraphPad Prism9.

Results: Our primary data demonstrates that SARS-CoV-2 spike specific IgG responses in convalescent pediatric patients decrease over time, especially at 6th month. IgG responses are significantly higher at 1st month in children with moderate disease severity compared to children with mild disease severity.

Conclusion: SARS-CoV-2 spike specific IgG levels in pediatric patients are shown to be decreased over time. However, even where IgG levels are at their lowest, they are still significantly higher compared to IgG levels of healthy control. With increasing disease severity and age, IgG levels, but not IgG1 nor IgG4, are increasing in pediatric patients. However, further studies are required to explain the levels of different types of immunoglobulin levels among children after COVID.

Acknowledgement

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Keywords: SARS-CoV-2, COVID-19, pediatric, antibody, IgG, IgG subtypes

Investigation Of Mitochondrial Metabolism Of *Helicobacter* Activated B Cells

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Objective: Mitochondria and immune cell functions are closely linked to each other. Studies have shown that high mitochondrial mass and membrane potential indicate active mitochondria. Shigella infection leads to dysfunctional mitochondria with loss of the mitochondrial membrane potential of human B cells for undergoing these cells to apoptosis. Nevertheless, mitochondrial metabolism of *Helicobacter*-stimulated B cells has not been elucidated yet. For this purpose, in our study, we investigated mitochondrial metabolism of *Helicobacter felis* (*H. felis*)- activated B cells.

Materials-Methods: We magnetically sorted B cells from the spleen of C57BL/6 mice and stimulated these cells with *H. felis* antigen (10 ug/ml), TLR2-ligand PAM3CSK4 (2.5 ug/ml) or TLR4-ligand LPS (10 ug/ml) for 6h, 24h, and 48h. Then, we assessed mitochondrial mass and membrane potential of B cells, using Mitoview Green and Mitoview 633 or TMRE dyes, respectively in flow cytometry. Additionally, we assessed the mtDNA (Cox1 gene)/nDNA(Rps18 gene) ratio and expression of Tfam with Q-PCR in order to investigate mitochondrial biogenesis.

Results: We found that stimulated B cells increased both mass and membrane potential of mitochondria by 20-30 % in 24h and 50-60% in 48h. But the mtDNA/nDNA ratio was decreased at 24h and 48h and Tfam expression was increased at 6h and 24h via different stimulations.

Conclusions: Activated B cells increased their active mitochondria. But at respective time points, B cells reduced their mtDNA number. Also, these cells increased Tfam expression indicating undergoing into mitochondrial biogenesis. Further studies are necessary to explain the changes in mitochondrial metabolism of these B cells in the presence of electron transport chain (ETC) inhibitors; oligomycin and rotenone.

Acknowledgments: This study was supported by the TUBITAK(Project Number: 119S447) and Istanbul Technical University, Department of Scientific Research Projects (ITU-BAP) (Project Number: 42944)

Keywords: Mitochondria, Immunometabolism, *Helicobacter*, B cells

The effect of glycolysis and OXPHOS inhibition on *Helicobacter* activated B cell's

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Objective: Immune cells activation, proliferation and functional state are known to correlate with their energetic metabolism. Studies have shown that inhibition of glycolysis and oxidative phosphorylation (OXPHOS) with inhibitors 2-DG and oligomycin suppresses B cell's proliferation, survival and function. However, the effect of glycolysis and mitochondrial inhibition on *Helicobacter*-activated B cells is unknown. In our study, we aim to show the impact of 2-DG and ATP synthase inhibitor oligomycin on *Helicobacter felis* (*H. felis*)-activated B cell's survival, proliferation and IL-10 secretion.

Materials-Methods: B cells were magnetically sorted from the spleen of C57BL/6 mice and stimulated with *H.felis* antigen (10 ug/ml), TLR2-ligand PAM3CSK4 (2.5 ug/ml) or TLR4-ligand LPS (10 ug/ml) in the presence or absence of 2-DG (0,1-0,5-1 mM) and oligomycin (0,1-0,5-1 nM) for 6h, 24h, and 72h. The viability of B cells was assessed with 7-AAD staining and CFSE was used for proliferation assay. Both viability and proliferation assays were analyzed by flow cytometry. IL-10 secretion level was investigated by IL-10 ELISA.

Results: Our results showed that higher doses of 2-DG decreased B cell viability and IL-10 secretion, which was most noticeable at 24h time point. 2-DG treatment suppressed B cell proliferation in all stimulation groups in a dose-dependent manner at 72h without causing significant cell death. On the other hand, oligomycin did not show any significant effect on either viability or IL-10 secretion.

Conclusion: Inhibition of glycolysis was more effective on B cell proliferation, survival, and IL-10 production compared to OXPHOS inhibition. These data suggest that the glycolytic pathway controls the expansion of *Helicobacter*-activated B cells and plays a critical role in IL-10 production from B cells. Further studies are necessary to understand the importance of glycolysis and OXPHOS inhibition in terms of regulatory B cell differentiation .

Acknowledgments:These studies are supported by TUBITAK-1001 Project 119S447-120S804 and ITU-BAP Project 43021

Keywords: B cells, *Helicobacter*, 2-DG(2-Deoxy-D-glucose), Oligomycin, Glycolysis, OXPHOS

[PP-51]

The Effect Of *Helicobacter*-Mediated Stimulation On Glucose Uptake, Intracellular Glucose Transporters, And Glycolysis Enzymes Levels In B Lymphocytes

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Objectives: B cells alter their metabolism in different ways during their activation and glucose is an essential molecule in the activation process to sustain biomolecule synthesis. The effect of B cell activation mediated by bacterial antigens on glucose metabolism has been previously shown for 24 hours, however, their effects at 6 and 48 hours are still unknown. Additionally, any *Helicobacter*-mediated stimulation effect on glucose metabolism has not been revealed on B cells. *Helicobacter*-mediated activation induces the differentiation of regulatory B cell subsets which play a crucial immunosuppressive role in autoimmunity and cancer prognosis. Therefore, in this study, we aimed to reveal *Helicobacter*-induced reprogramming of glucose metabolism in B cells.

Materials and Methods: We magnetically sorted B cells from the spleen of C57BL/6 mouse and incubated them with either *H.felis* antigen (10 ug/ml) or Pam3CKS4 (2.5 ug/ml) (TLR-2 ligand) or LPS (10 ug/ml) for 6h, 24 h, and 48h. We measured the uptake level of fluorescent-labeled glucose analog, 2-NBDG, in live B cells by flow cytometry in addition to assessing intracellular levels of glucose transporters; GLUT1, and GLUT3, at specified time points. mRNA levels of Glut1, Glut3, Pkm2, and Ldha over 48 hours were measured by Q-PCR using Sybr-based method.

Results and Conclusions: Our results demonstrated that the *H.felis* antigen can lead to metabolic reprogramming via increasing glucose uptake in B cells. The 2-NBDG uptake level was increased similar to earlier studies related to LPS stimulation. In previous studies, GLUT3 was shown to be downregulated, and GLUT1 was shown to be upregulated which suggested to be an essential protein among transporters to support B cells. However, our results showed that Glut3 levels were increased similar to Glut1 in all conditions. Expression levels of Glut1, Pkm2, and Ldha was increased but Glut3 was decreased at 24 hours.

Acknowledgement: These studies are supported by TUBITAK-1001 Project, 119S447 and ITU-BAP Project, 43020

Keywords: B cell, *H.felis*, Glucose, Glucose Transporters, Glycolysis Enzymes

Investigating SARS-CoV-2 nucleocapsid-specific IgG antibody levels in pediatric convalescent COVID-19 patients

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Objective: Coronavirus disease 2019 (COVID-19) had an enormous impact on the world. Early reports indicated that the immune response of children to COVID-19 differs from adults. Thus, it is worth investigating the differences in the immune response of children. One major component of adaptive immunity to infection is antibody production, hence we investigated the antibody production in children after having COVID-19. We focused on IgG antibodies, the SARS-CoV-2 nucleocapsid-specific IgG levels were tested in COVID-19 convalescent children at 4 different time points (1st month, 3rd month, 6th month, and 9th month post-COVID).

Materials-Methods: Patients who participated in this study were aged between 18 months to 18 years, and all patients were confirmed COVID-positive via a PCR test. Children without showing any symptoms were considered as healthy control. Peripheral blood of the participants was collected at different time points (1, 3, 6, and 9 months- post-COVID). SARS-CoV-2 nucleocapsid protein-specific IgG levels were assessed using the indirect ELISA method.

Results: Our preliminary results showed that in the 1st, 3rd, and 6th-month post-COVID, children had significantly higher SARS-CoV-2 nucleocapsid-specific IgG levels compared to the control group while in the 9th month time point the IgG level was not significant. We also observed that 3rd-month post-COVID children with mild disease severity had significantly higher nucleocapsid-specific IgG levels compared to 6th and 9th-month mild post-COVID cases.

Conclusion: Higher SARS-CoV-2 nucleocapsid-specific IgG levels were detected in children at the 1st, 3rd and 6th month post-COVID. As more time passes, the detectable amount of nucleocapsid-specific IgG in peripheral blood in convalescent children tends to decrease. The correlation between age groups, disease severity, and hospitalization status to nucleocapsid-specific IgG should be investigated in further studies.

Acknowledgements: This project is funded by TUBITAK (Scientific and Technological Research Council of Turkey) 1001 Project (Project number: 120S804).

Keywords: COVID-19, SARS-CoV-2, nucleocapsid-specific IgG, pediatric

Helicobacter Pylori Virulence Factors and T-Cell Cytokines in Helicobacter pylori-Infected Pediatrics Patients with Gastritis

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Background: More than half of the world's population and more than 75% of Turkey's population are infected with *Helicobacter pylori* (*H.pylori*), a gram-negative, microaerophilic spiral, motile pathogen. *H. pylori* infection can cause peptic ulcers, gastritis, and gastritis cancer. Adults' clinical outcomes of infection have demonstrated a significant correlation between *H. pylori* infection and bacterial virulence factors. The relationship between childhood infection and virulence factors has not been extensively investigated. The aim of this study is to investigate the relationship between several virulence genes that take a role in bacterial infection in *H. pylori*-infected pediatric patients and determining CD4⁺ T-cell response. These virulence genes are ureA-ureB, vacA, dupA, napA, hpaA, sabA, oipA, iceA1A2, GGT.

Materials-Methods: During the study, antrum biopsies were obtained from 80 children with gastritis, aged 5-18 years, who applied to Sariyer Hamidiye Etfal Hospital. DNA isolation and RNA isolation from the biopsy samples performed. The presence of *H. pylori* was detected by urease PCR. Conventional PCR was used to examine 25 *H. pylori* positive strains for the presence of the vacAs1s2, vacAm1m2, dupA, napA, oipA, hpaA, sabA, iceA1A2, GGT genes. The relationship between virulence factors was evaluated using Pearson product moment correlation coefficient. Additionally, IL-17, FOXP3, IFN- γ mRNA levels were determined by RT-PCR and analyzed by student-t test.

Results: A significant negative correlation between vacAs1 and vacAs2 was found in our study. Moreover, a significant positive correlation was reported between vacAs1-napA, vacAs1-sabA, vacAs1-iceA1, dupA-napA, dupA-sabA, dupA-iceA1, dupA-ureB, napA-oipA, napA-sabA, napA-iceA2, oipA-iceA1, oipA-iceA2, oipA-ureB, oipA-GGT, sabA-iceA2, sabA-ureB, iceA1-ureB, iceA2-ureB, iceA2- GGT and ureB- GGT. In children with *H. pylori* (+), IL-17 and IFN- γ mRNA level was significantly higher, whereas FOXP3 level was significantly lower in *H. pylori* (-).

Conclusion: Our data suggest that significant correlation between virulence factors in different H.pylori strains. We will focus on the correlation between virulence genes of H.pylori and CD4+ T cell response in our pediatric patients in future studies.

Acknowledgement: This study was supported by Turkish Pediatric Gastroenterology Hepatology and Nutrition Association, ITU-BAP-43556.

Keywords: Helicobacter Pylori, H.pylori virulence factors, gastritis, immune response, Turkish pediatric population

NEUTROPHILS AND LOW(ER)-DENSITY NEUTROPHILS (Ldns) İn COVID-19

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Introduction: Neutrophils are key players in innate immune responses and their maturation stage affect these cells phenotypic and functional differences. In this study, the interaction between neutrophils maturation stage and COVID-19 prognosis was evaluated.

Material-Methods: Low and high density of neutrophils that were accumulated both upper and lower phase of 1.077g/mL Ficoll phase leukocytes were obtained from upper or lower 1.077g/mL ficoll phase of COVID-19 positive patients (mild, moderate, severe disease) and healthy donors. High and low density neutrophils were positively selected using with CD66b microbeads by magnetic activated cell sorting. Different CD66b surface expression levels were detected (dim, mod and high) and purified by floresen activating cell sorting (FACS). These subpopulations surface molecules expression (CD80,CD86,PD-L1, PD-L2,HLA-DR,CD117,CD10,CD63, CD62L,CD114 ve LOX- 1) were studied with flow cytometry.Dichlorodihydrofluorescein diacetate(DCFDA) was used to measure ROS production.Carboxyfluorescein succinimidyl ester(CFSE) for proliferation analysis of CD4+ or CD8+ T cells obtained from healthy donors in the presence of low or high density of neutrophils from patients.

Results and Discussion: CD63+ low density neutrophils was higher in the 1.077g/mL ficoll fraction from patient with mild disease, compared with asymptomatic. Severe disease high density neutrophils' CD117 expression was lower than mild disease. Low density neutrophils' CD114 expression was found higher in asymptomatic patients than severe. Interestingly, immature and suppressive character marker LOX-1 was found high amount of both upper and lower 1.077g/ mL ficoll phase of COVID-19 positive severe patients. This study is being supported by The Scientific and Technological Research Council of Turkey (TUBITAK), Project no. 120S653

Keywords: COVID-19, neutrophils, neutrophil sub-groups, low density neutrophil

Effects Of Paclitaxel (Pax) And Nanobubble Ozone Stored Niosomes (NOSN) Combination On 4T1 Breast Cancer Related Immune Responses

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Introduction: Anti-tumor treatment approaches, chemotherapy currently primary treatment application though; minimal toxicity, anti-tumor and reinforcing immune response different treatment strategies is sought. In this study, paclitaxel (Pax) and non-toxic, reason for high penetration feature nanobubble ozone stored niosomes (NOSN) combined anti-tumor therapy and immunomodulator effects were evaluated.

Material-Methods: NOSN, (1.25,0.6,0.3,0.15,0.078,0.114,0.057%) and Pax, (2.5,5,10,20,40,80ug/mL) IC50 and ED50 values were evaluated in Raw 264.7, L929, 4T1 and EMT6 cell lines by MTT method. Incubations were evaluated by MTT method at 24, 48, 72h. Determined Pax and NOSN concentrations/percentages were added to 4T1 and EMT cells alone and/or in combination and cell viability was evaluated by MTT method. In immune response experiments, after cultures, mouse splenocytes were labeled with Carboxyfluorescein succinimidyl ester (CFSE) and co-cultured in the presence of anti-mouse CD3 at a ratio of 0.125:1,0.25:1,0.5:1,1:1,2:1 (cancer cells:splenocytes) for 72h. At the end of the incubation, splenocytes were stained with CD4, CD8, CD25, FoxP3 and CD4+ or CD8+T cell proliferation and regulatory T cell (Treg) transformation were evaluated by flow cytometry method.

Results and Discussion: IC30 value (70% viability) for the Ozon formulation (NOSN) was determined as 0.078%. In breast cancer cell lines, NPLO 0.057% and 0.114%; Pax 2.5 and 5ug/mL were cultured alone and/or with combination. As a result of 5ug/mL Pax+0.114% NOSN treatment in 4T1 cells, viability was detected as 15%. When the remaining cells were cultured with splenocytes, the percentage of CD4+CD25+FoxP3+Treg cells was observed 1.1%. Compared with all conditions of EMT6 and 4T1, this condition is thought to be an approach that supports both anti-cancer and immune response, and will be supported by in vivo studies.

Keywords: Paclitaxel, nanobubble ozone stored niosomes, anti-tumor therapy, Treg, 4T1 breast cancer

The effects of Regnase-1 in sculpting the tumor microenvironment

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Objective: CAFs induce the accumulation of monocytes in the tumor-microenvironment and their differentiation into M2-like-macrophages. The mechanisms underlying monocyte-accumulation in the tumor *milieu* by CAFs are not clear. Therefore, we aimed to determine the role of CAFs in monocyte-recruitment and macrophage-polarization in the tumor-microenvironment. Moreover, we investigated the effects of Regnase-1, which is induced by MCP-1 (a critical regulator of monocyte-accumulation) on monocyte-recruitment by CAFs.

Materials-Methods: CAFs were isolated from patients with breast tumor using Collagenase-I (1 mg/mL) and Hyaluronidase (125 U / mL) followed by differential-sedimentation and plating. Characterizations of fibroblasts were performed by immunocytochemistry stainings with vimentin, pan-cytokeratin and α -SMA. Normal human-fibroblast-cells (MRC-5) were utilized as the control-group. Expression levels of Regnase-1 and other related proteins were investigated by Western blot analyses.

Results: 4 patients included in the study had the diagnosis of infiltrative-ductal-carcinoma, while 1 patient had the diagnosis of invasive-lobular-carcinoma. Our results demonstrated that CAFs expressed α -SMA, while normal-fibroblasts did not. We evaluated the correlations between the patients' clinical and pathological features with CAF-grade of the isolated fibroblasts. More than 60% of α -SMA positivity correlated with higher Ki-67 index. Western blot analyses demonstrated that CAFs obtained from breast tumors expressed Regnase-1 and MCP-1 proteins. The protein expression of MCP-1 was higher in fibroblasts that demonstrated higher α -SMA expression among CAFs. Moreover, CAFs with higher α -SMA expression showed higher expression of cleaved Regnase-1 (50 kDa). Spearman's correlation analysis revealed that there was a significant and strong correlation between MCP-1 and cleaved Regnase-1 expressions ($p < 0.05$). Last but not least, we demonstrated that CAF-grade was also correlated with protein expressions of MCP-1 and Regnase-1 ($p < 0.05$).

Conclusions: MCP-1 and Regnase-1 (Monocyte-chemoattractant-protein-induced-protein-1) might be implicated in macrophage-polarization in the tumor-microenvironment. The identification of molecules driving macrophage-plasticity in the cancer-microenvironment could provide a basis for macrophage-focused theranostic strategies.

Keywords: breast cancer, CAF, monocytes/macrophages, Regnase-1, MCP-1, tumor microenvironment

[PP-58]

Investigation of pre-clinical and phase II clinical studies of VLP-58-1023-AL-K3-PII vaccine for Alpha variant

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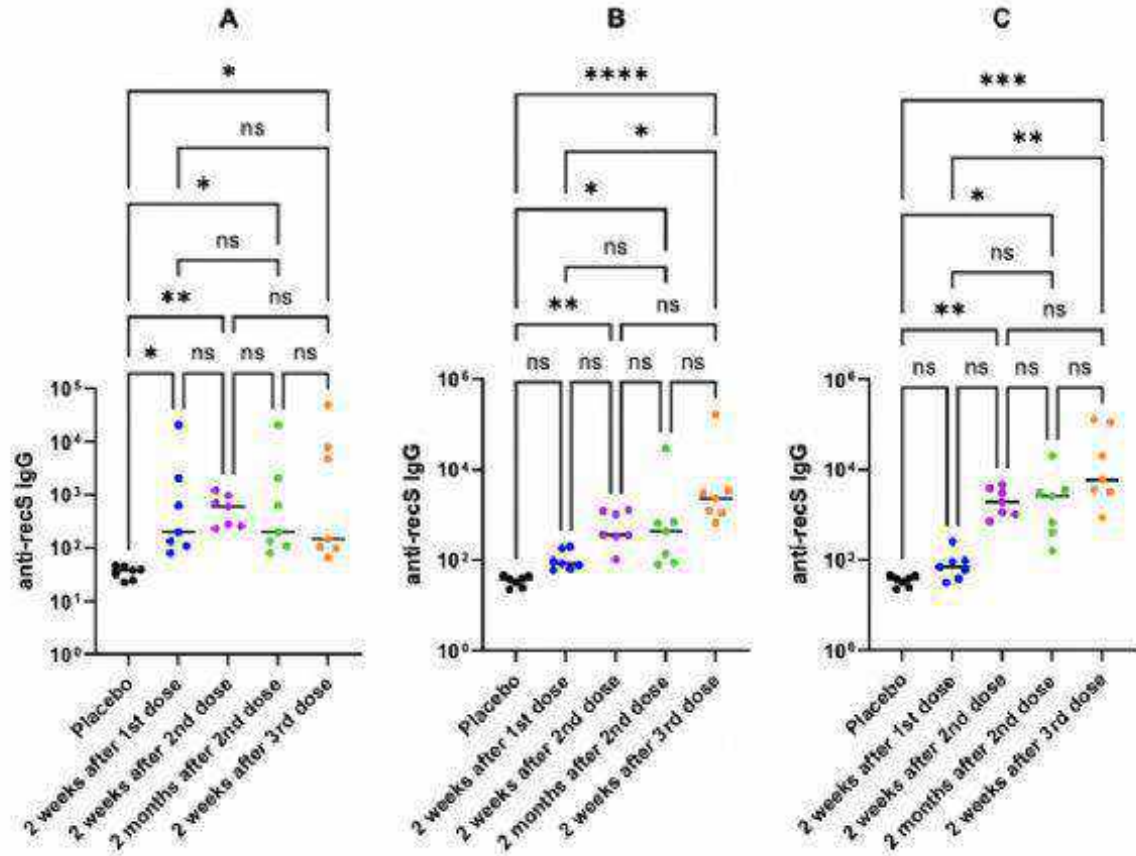
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In December 2019, SARS-CoV-2 was discovered in China and described as the causative agent of COVID-19. The disease has spread rapidly across the world and has been declared a pandemic by the World Health Organization. The development of an effective vaccine has become the most significant issue to constrain the pandemic. Most of the current vaccines used only Spike as antigen to generate antibodies for preventing the virus entry and replication. However, concerns have raised about Spike-based vaccines with the emerging of variants as they can moderately escape from neutralizing antibodies. So, we developed Virus-like particle vaccine which displays hexaprotine prefusion-stabilized Spike, N, M, E proteins, and adjuvanted with Alum and K3-CpG ODN. We preferred to use Alpha variant because of its high mortality risk and selection advantages. First, we designed three different vaccine formulations and according to antibody responses in mice we determined the optimal formulation and dosage for human use. Pre-clinical studies revealed that the best vaccine combination was high dose antigen and low dose adjuvants. We determined the effects of third dose administration in mice so that its applicability to humans could be determined. It was found that third dose injection increased the antibody levels much higher and provides sustained immunity. Both humoral and cellular immune responses of Phase II clinical trial volunteers were studied. ELISA experiments indicated that VLP-58-1023-AL-K3-PII vaccine induced great amount of humoral immune responses against S,N proteins and WT, Alpha, Delta RBDs. All cytokine levels specific to SARS-CoV-2 peptides demonstrated that the vaccine elicited Th1-biased responses. Taken together, this study revealed that VLP-58-1023- AL-K3-PII vaccine for Alpha variant successfully elicited both humoral and cellular immune responses, its effectiveness against other variants was indicated and the efficiency of the vaccine could be increased with the administration of third dose.

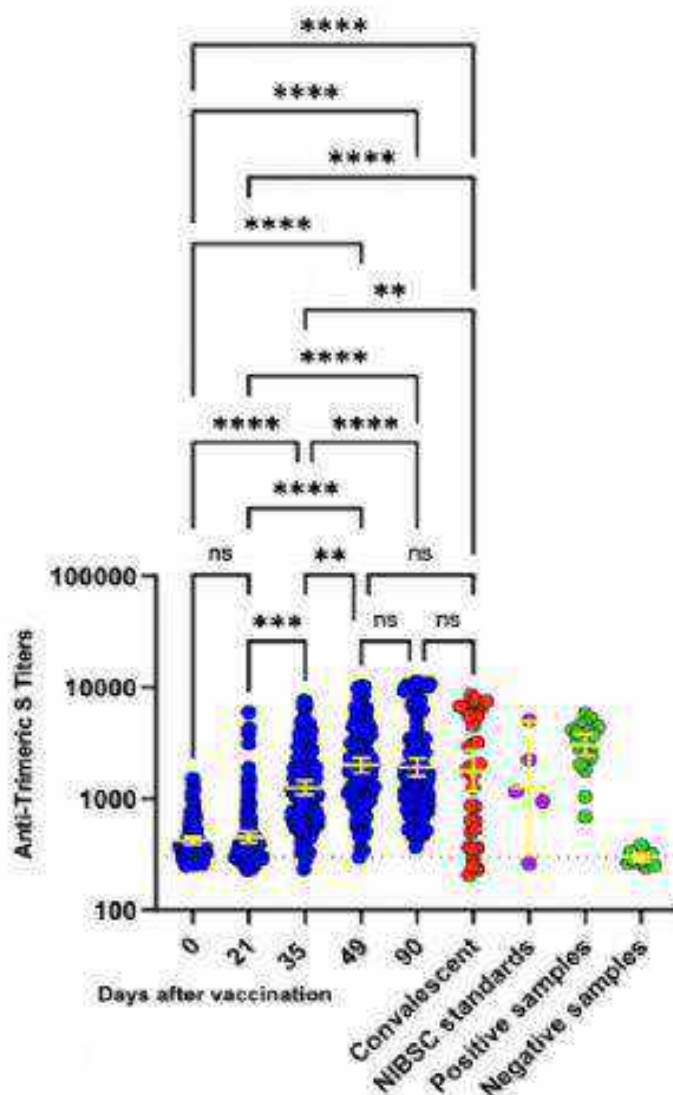
Keywords: SARS-CoV-2, Virus-like particles, vaccines, CpG ODN, Alum, humoral and cellular immunity

Evaluation of IgG antibody levels of mice against in-house trimeric S protein.



ELISA plates were coated with in-house trimeric S protein and IgG antibody production of mice ($N=7/\text{group}$) sera from 2 weeks after 1st dose, 2 weeks after 2nd dose, 2 months after 2nd dose and 2 weeks after 3rd dose were measured. Placebo group were used as a negative control. Comparison of the IgG responses at indicated time points was done using one-way ANOVA with Dunnett's multiple comparisons test. (ns: non-significant, $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$, $****p \leq 0.0001$.) (A: 2.5:120:60, VLP:Alum:CpG, B: 5:120:60, C: 5:60:30)

Measurement of anti-Spike IgG responses of vaccinated volunteers and comparison with convalescent sera.



	0	21	35	49	90	Convalescent
Number of values	115	115	115	114	106	35
Geometric mean	418.4	449.1	1248	1993	1903	1756
Geometric SD factor	1.505	1.849	2.239	2.317	2.635	3.485
Lower 95% CI of geo. mean	388.0	400.9	1075	1705	1579	1143
Upper 95% CI of geo. mean	451.2	503.0	1448	2330	2294	2696

ELISA plates were coated with in-house trimeric S protein and IgG antibody production of serum samples of volunteers from Day:0, 21, 35, 49 and 90 and convalescent sera (N=35) along with NIBSC samples (N=37) and NIBSC standards (N=5) were measured. Comparison of the IgG responses at indicated time points was done using one-way ANOVA with Dunnett's multiple comparisons test.(ns:non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.)

Therapeutic Use of Bone Marrow-Derived Tolerogenic Dendritic Cells Modified by Lentiviral Transduction in C57BL/6 EAE Model

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Introduction: Multiple sclerosis (MS), which is one of the chronic autoimmune neurodegenerative diseases, is the most common cause of disability in young adults all over the world. As with a variety of autoimmune disorders, there is evidence of an altered level of tryptophan catabolism in MS patients. Indolamine-2,3-dioxygenase (IDO), the enzyme that initiates the breakdown of tryptophan, plays an important role in MS. On the other hand, activation of T lymphocytes requires recognition of the peptide-major histocompatibility complex and co-stimulatory signals provided by antigen-presenting cells. T cell activation without co-stimulus can lead to anergy.

Method: In the first strategy, DCs were modified with lentiviral vector encoding only expression of IDO, which catabolizes tryptophan. In the second approach, DCs with a lentiviral vector encoding a fusion protein called CTLA4-KDEL that inhibits the expression of B7 (CD80/86) molecules on the cell surface by keeping co-stimulatory molecules in the endoplasmic reticulum. In the third approach, DCs with a lentiviral vector encoding IDO overexpression and silencing with shRNA CD80/CD86 in the same vector construct. Genetically modified DCs were administered intravenously to EAE model mice. Treated EAE model mice were followed for two weeks. Finally, histopathological examination was performed to evaluate the lesions in the central nervous system. In addition, the brain and cerebellum were analyzed histochemically in order to observe demyelination that may cause damage to the protective covering (myelin sheath) that surrounds nerve fibers in brain.

RESULTS

We showed that downregulation of CD80 and CD86 expression alone or in DCs overexpressing IDO resulted in reduced clinical scores with these treatment strategies. However, the most effective treatment approach is the use of genetically modified overexpressed IDO and downregulated (CD80/CD86) in the same construct. Tolerance-inducing dendritic cells have

exciting potential for the treatment of MS and other autoimmune diseases. This study is being supported by TUBITAK(118S474)

Keywords: Multiple Sclerosis, Dendritic cells, indoleamine-2, 3-dioxygenase, Lentiviral Transduction, CD80/86

Determination of Functional Alterations in Type-1 Helper T (Th1) Cells Under Radiation Stress

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Radiation therapy is an important component of cancer treatment, nevertheless T cells in the tumor mass are also targeted by radiation. Therefore, it is critical to understand the functional behavior and viability of T cells which might be in distinct activation phases. Within the scope of this study, CD4⁺ T cells were stimulated by anti-CD3 monoclonal antibody and monocytic cell line-derived co-stimulation resulting in distinct functional phases such as early activation, effector and exhaustion. Different radiation doses (0 Gy, 2 Gy, 5 Gy, 8 Gy, and 12 Gy) were applied on the T cells obtained at distinct periods (0, 24, 72, and 120 hours) of stimulation. Cell viability, DNA break points, mitochondrial membrane potential, IL-2, IFN- γ , and TNF- α secretion, proliferation and exhaustion phenotype were assessed by flow cytometry, immunofluorescence, and cytological techniques. The cells were most radiosensitive at the effector phase (72h), especially the effector memory-like T lymphocytes. The most radiation-resistant subtypes were naive T and central memory-like T lymphocytes. It was observed that radiation did not change the mitochondrial membrane potential in Th cells. It was shown that anti-PD-1 blockade applied before radiation exposure had no negative impact on Th1 survival. As a result, it was determined that Th1 cells did not show radiation sensitivity in the process of regaining function with PD-1 blockade.

Keywords: T lymphocyte activation, CD4⁺ T cell, radioimmunotherapy, γ -H2AX, mitochondrial membrane potential

Transcriptomic characterization of TCR-NK cells reveals challenges associated with CD3 ζ overexpression

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Objective: Our group has previously shown the proof-of-concept for NK cells genetically modified to express functional T-Cell Receptors. NK cells expressing CD3 δ ,CD3 γ ,CD3 ϵ and TCR α/β on their cell surface can detect an antigenic peptide presented by MHC-I and selectively show antigen-specific cytotoxic activity. The overexpression of CD3 ζ in TCR-NK cells doesn't seem to increase antigen-specific triggering but shows increased background activity against non-specific targets. However, mechanisms of increased non-specific activity caused by CD3 ζ overexpression is unknown. Therefore, we aimed to characterize the gene expression profiles of TCR-NK cells by bulk RNA sequencing to understand the transcriptomic changes associated with TCR modification of NK cells.

Materials-Methods: NK92 cells were genetically modified by using lentiviral constructs to express TCR on their surface. CD3 complex (CD3 δ ,CD3 γ ,CD3 ϵ) with or without CD3 ζ and TCR α/β genes were transduced consecutively and pure populations of cells were obtained. Genetically modified cells were synchronized, RNA-isolation was performed, and followed by bulk RNA sequencing. Differentially Expressed Genes were identified with R:limma and statistical analyses were done with R scripts.

Results: Our analysis shows that genes which have roles in viral defense mechanism pathways are significantly altered in all genetically modified cells as expected, due to the side-effects of gene delivery by lentiviral vectors. Apart from these genes, introduction of CD3 complex (without CD3 ζ) and TCR α/β didn't cause many significant changes. On the other hand, more than 600 genes were significantly affected by inclusion of CD3 ζ during TCR-NK cell generation. These alterations are potentially linked to the increase in non-specific activity of TCR-NK cells that overexpress CD3 ζ .

Conclusion: The inclusion of CD3 ζ in vector design may be deleterious to the function of TCR in NK cells due to the dramatic changes in NK cell gene expression profile. Further development of TCR-NK cells should rely on expression of only CD3 δ ,CD3 γ ,CD3 ϵ components of the CD3 complex.

Keywords: T-Cell Receptor, NK cells, TCR-NK

Investigation of Pre-clinical and Clinical Results Against SARS-CoV-2 Wild-Type and Alpha Variants Combination for VLP-58-1023-AL-K3 Vaccine

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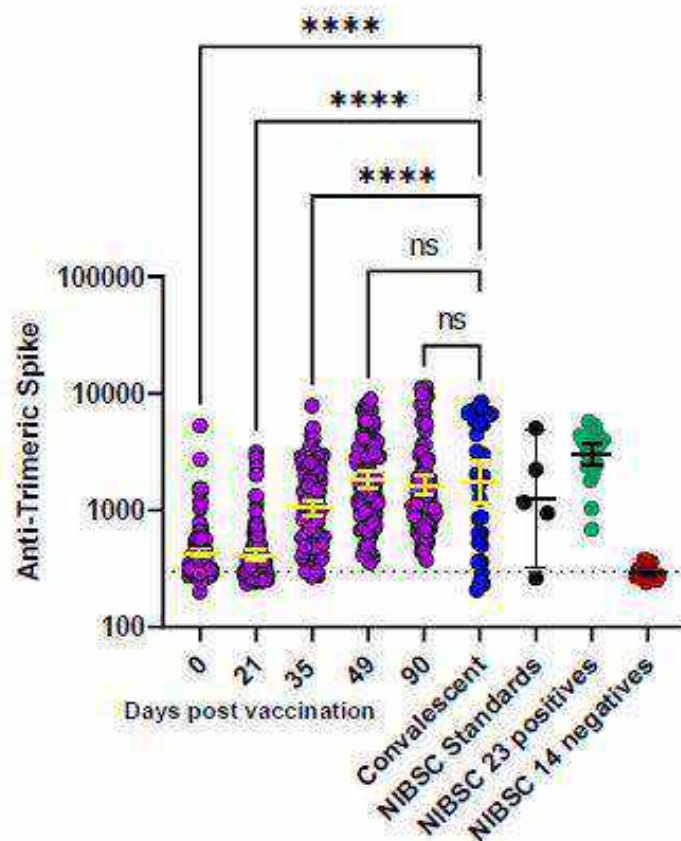
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The SARS-CoV-2 had created need of suitable vaccine candidates' development with its quick spread. Most of these vaccines are based on the Spike protein, which allows the virus to enter the host and survive. Although the Spike protein is effective in the formation of neutralizing antibodies in the person, the virus essentially has 4 structural proteins, not only spike. Our vaccine strategy containing VLP (virus-like particles) was based on the self-assembly property of Spike, Membrane, Envelope and Nucleocapsid proteins, and these protein structures were transformed into plasmids with his-tag labels, and the products were collected in mammalian cells in vitro by the transfection method. VLPs were purified by affinity chromatography and formulated with Alum and CpG adjuvants. Since the virus is open to new mutations over spreading, it is very important that our vaccine should be editable and adaptable to new virus variants. Due to the alpha variant effect in the world, which has become widespread all over the world recent years, WT VLP formed with a more stable Spike protein containing a thermostable sub-proline mutation was used in the first dose of our vaccine, but UK VLP containing alpha variant mutations was used in the second dose. Aluminum and CpG oligodeoxynucleotide adjuvants were used together for both injection formulations. Our vaccine has successfully completed Phase 2 studies. As a result of these studies, approximately 115 vaccinated volunteers were followed up for 90 days and immunological analyzes were performed with samples taken at certain day intervals. As a result of these analyzes, moderate and high levels of neutralizing and non-neutralizing antibody responses were observed in many patients, and a humoral immune response was induced until day 90. In addition, it was observed that the cellular responses of the volunteers are progressed in tendency with the T helper 1 cell response.

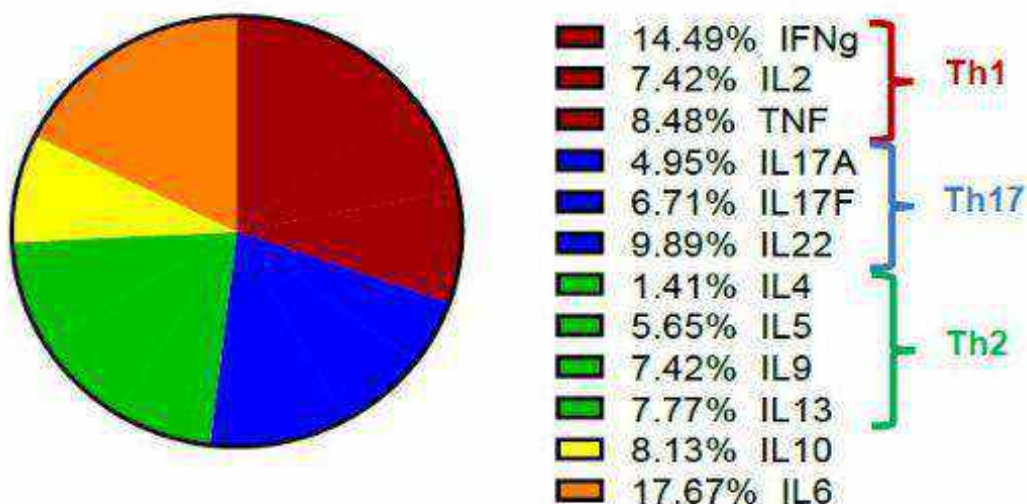
Keywords: COVID-19, SARS-CoV-2, VLP, Vaccine, CpG ODN, Alum

Antibody Production of Phase 2 volunteers against in house recombinant trimeric Spike Protein



Collective dot graphs of all Phase2 volunteers in regular day intervals after first injection of VLP-58-1023-AL-K3 vaccine (0,21,35,49,90) against spike with the comparison of convalescents sera, NIBSC standards and NIBSC convalescent plasma samples (positive and negative plasma); 96-well 2HB maxi-binding ELISA plates were coated with 6 µg/ml in-house recombinant spike protein and plates were blocked. After the serial dilutions of volunteers' sera, ALP-tagged anti-human IgG secondary antibody were filled into wells and finally PNPP substrate was added into plate and optical density values were recorded at 405 nm. Number of values and statistical values were indicated in the table below the figure. The comparison was done by one-way ANOVA with Dunnett's multiple comparisons test. $P < .05$, $**P < .01$, $*P < .001$, $***P < .0001$.

T response pie chart of Phase 2 volunteers



Collective pie chart of Phase2 volunteers to show cytokine production belong to all T responses after stimulation of PBMCs with S, N, M, O peptide pool. Red part shows Th1 responses with indicated percentages of IFN- γ , IL-2 and TNF- α . Blue part shows Th17 responses with indicated percentages of IL-17A, IL-17F and IL-22. Green part shows Th2 responses with indicated percentages of IL-4, IL-5, IL-9 and IL-13. Yellow part shows Treg response with indicated percentage of IL-10. Orange part shows IL-6 production.

Formulation of VLP vaccine for Phase 2 study

1ml injection	40 μ g VLP + 600 μ g Alum + 300 μ g CpG ODN-K3	1st injection WT VLP (Day0)	2nd injection UK VLP (Day21)
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Necessities to be a volunteer

INCLUSIONS	EXCLUSIONS
Female and/or Male	-
Ages between 18-59	Ages under 18, above 59
Negative for SARS-COV2 PCR test	Positive for SARS-COV2 PCR test
Willingness	Reluctance
Body Mass Index (BMI) between 18-35 kg/m ² .	Body Mass Index (BMI) under 18 or above 35 kg/m ²
Physical and psychological health	Unhealthiness

Immunomodulatory Effect Of *Cephalaria Speciosa* Saponins On Macrophage Cells Against IBV D 274 Antigen

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Recent studies in the field of immunology indicate that immunizations are now more purposefully utilized to achieve specific, protective, and long-lasting immunity. The judicious selection of adjuvants may be influenced by the sort of immunological response which is required. The essential cellular components of innate immunity and macrophages have great plasticity and may distinguish the abnormality in response to a range of stimuli. Due to this, macrophages show notable variation in both diseased and healthy environments. Considering the importance of macrophages in pathogen recognition, phagocytosis, processing, and antigen presentation, this study aims to examine the impact of *Cephalaria speciosa* (CSP) molecules on the different phenotypes and functions of the macrophage-like cells derived from THP-1 monocytes. For this purpose, THP-1 cells were differentiated into macrophage-like cells by PMA [Phorbol 12-myristate 13-acetate], and then cultured with CSP molecules with or without IBV D 274 antigen. CD 11b, CD 80, and CD 163 antigens were used to determine the macrophage polarization. The Mean Fluorescence Intensity of CD 11b, CD80, and CD 163 were analyzed on a flow cytometer. The results showed that CSP molecules caused both M1 and M2 polarization in THP-1 macrophages. Notably, the polarization of M2 in comparison with M1 was significantly higher. It was noted that the CSP molecules cause Th2 to be released from M0 macrophages, which in turn promotes the polarization of M2 macrophages. Moreover, applying an antigen with CSP molecules results in a rise in macrophage polarization, which will eventually enhance the immune system response. In conclusion, by promoting M2 macrophage polarization from THP-1 cells, CSP molecules can be suggested as promising stimulators for Th2 cells which can activate and maintain the humoral or antibody-mediated immune response against extracellular parasites, bacteria, allergens, and toxins.

Keywords: *Cephalaria speciosa*, saponin, Macrophage, M1, M2

Investigation of Inflammasome Activation in Response to SARS-CoV-2 Virus

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Background. The emerging SARS-CoV-2 virus is responsible of the Covid-19 pandemic. Some patients with severe Covid-19 experience cytokine storm characterized by an uncontrolled secretion of several cytokines. Inflammasomes are innate immune receptor that recognize different pathogens and trigger inflammation through the secretion of the pro-inflammatory cytokine IL-1beta and the induction of a pyroptotic cell death.

Objectives. Although the role of inflammasomes in sterile and bacterial inflammation is well known, their involvement in virus detection and elimination is less understood. Recent publications suggest that SARS-CoV-2 infection activates the NLRP3 and AIM2 inflammasomes. However, the molecular mechanism of this activation is unclear. This study aimed to determine which SARS-CoV-2 protein(s) activate(s) inflammasomes.

Materials and Methods. Different SARS-CoV-2 proteins were screened for inflammasome activation in two experimental systems. Firstly, the inflammasome complex was artificially reconstructed in human fibroblast HEK293FT cells co-transfected with viral proteins individually and IL-1beta activation in response to viral protein expression was monitored by western blotting. Secondly, inflammasome oligomerization was assessed by ASC speck quantification in HEK293FT-ASC-GFP cells transfected with different viral proteins. Furthermore, viral proteins were tested for inflammasome receptor binding by molecular docking.

Results. Screening of SARS-CoV-2 proteins showed that besides the already published ORF3a protein, another viral protein activates IL-1beta cleavage in HEK293FT cells transfected with NLRP3, ASC, pro-Caspase-1 and pro-IL-1beta. When transfected into HEK293FT cells, this protein also induced a statistically significantly higher number of ASC speck compared to the mock transfected control. Molecular docking analyses revealed that this viral protein interacts with the inflammasome receptor.

Conclusion. Altogether, these results indicate that a new SARS-CoV-2 protein activates inflammasome oligomerization leading to IL-1beta activation and secretion in our experimental setting. These findings may contribute to the understanding of the molecular mechanism of Covid-19 and the development of efficient therapies by targeting this protein.

Keywords: SARS-CoV-2, Covid-19, Inflammasomes, IL-1beta, Molecular docking

Allergen-specific immunotherapy induces regulatory B cell subsets in patients with allergic rhinitis

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Introduction: Allergic rhinitis (AR) is an immune globulin (Ig) E-mediated inflammatory disease triggered by inhalation of seasonal or perennial allergens. Ceasing of conventional pharmacotherapies could lead to disease relapse, therefore, allergen-specific immunotherapy stands as the solitary therapy option for tolerance induction, which consequently helps for a long-term cure. Regulatory T (Treg) cells following AIT were revealed to contribute in maintenance of allergen tolerance but the role of regulatory B (Breg) cells still need illumination. This study aimed to investigate Breg subsets following long-term AIT with a clinical success.

Method: The patients enrolled to this study (25 new diagnosed patients with AR, 25 patients with long-term (32.52 ± 5.25 months) house-dust mite specific sub-cutaneous allergen immunotherapy (SCIT) and 25 healthy controls) were followed by Istanbul University, Istanbul Faculty of Medicine. Lymphocyte subsets were determined with a direct staining protocol in anticoagulated fresh blood samples. CD19⁺CD25⁺CD71⁺CD73⁻ and CD19⁺CD24^{hi}CD27⁺ Breg cell subsets were investigated.

Results: Allergen specific immunotherapy resulted with increased CD19⁺CD25⁺CD71⁺CD73⁻ subset. The lowest levels of this subset were detected in healthy controls. CD19⁺CD24^{hi}CD27⁺ subset was also significantly increased following AIT, however with the lowest content in AR patients.

Discussion: The initial results of this study revealed induction of both Breg cell subsets in AR patients as a consequence of a successful AIT. The different expression patterns of these cell subsets may be due to differential regulatory roles in healthy individuals and also in patients, which should further be investigated.

Keywords: Allergen specific immunotherapy, allergic rhinitis, B cells, immunotherapy, tolerance

The Effects of Progesterone on mPRa/nPR signaling in Triple Negative Breast Cancer (TNBC) Associated Immune Responses

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Introduction: Triple-Negative Breast Cancer(TNBC),is clinically negative for estrogen and progesterone receptor expressions and HER2 amplification.Progesterone exerts its effects via two different types of receptors; one of them is nuclear receptors(PR) and the other is the membrane progesterone receptors(mPR),which were discovered later.In TNBC, the nPRs are absent or expressed barely. Currently,despite this knowledge;the discovery of the effects of progesterone on several molecular parameters in TNBC raised the questions whether mPR signaling could be involved in this type of cancer.In this study,we focus progesterone effect on TNBC and immune cells and this interaction effect on immune responses

Material-Methods: 4T1,EMT6,4T1C(lentiviral modified,mPR was silenced by our group),4TS02 (lentiviral modified, scrambled) BALB/c mice derived triple-negative breast cancer cell lines were used.Mononuclear cells and all leukocytes isolated from spleen and bone marrow.CD11b+ cells were purified from bone marrow and spleen to perform myeloid cells-cancer cells co-cultures.Raw264.7 cell line was selected as a macrophage. Cancer cells and these immune cells incubated(24h,48h,72h,6Days) w/wo different progesterone concentration(0.8,6.4,12.8uM); mifepristone(MF,nPR antagonist).At the end of incubation cells were stained with anti-mouse-CD45 and CD45-cancer cells immune primed) and CD45+immune cells(cancer-cell primed) were purified from co-culture by FACS.These cells co-cultured with CFSE labeled splenocytes in the presence of anti-mouse-CD3. CD4+andCD8+T cell proliferation was evaluated by flow cytometry.CD80,CD86,CD11b,CD206, Ly6G, Ly6C were studied with flow cytometry for monocyte/macrophage activation and/or polarization. MTT assay was used for determination of the effects of progesterone on cell viability and proliferation.Purified cancer cell subpopulation from co-cultures were plated for scratch assays and analyzed with ImageJ. **Results and Discussion:** In this study,our results indicate that progesterone supports anti-cancer immune responses via mPR.These preliminary data are compared with the results obtained from in vivo experiments and evaluated holistically.Thus,the direction of the relevant immune response and the cells through which the mechanism takes place will be elucidated. Supported by TUBITAK,Project.no.219S666

Keywords: Triple-Negative Breast Cancer, progesterone, nPR, mPR, anti-cancer immune responses

[PP-73]

The effects of miR200a and TFAM Expression Levels on Function of Pancreatic Cancer Cells

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Pancreatic cancer is an aggressive malignancy with a poor prognosis. Early diagnosis and application of tumor-specific treatment are critical in pancreatic cancer. Therefore, new biomarkers are needed in the diagnosis and treatment of tumors. Also, miRNAs cause mRNA degradation and translational inhibition of targeted genes and play essential roles in controlling gene expression. miR-200a-3p acts as a tumor suppressor and one of its target genes is Mitochondrial Transcription Factor A (TFAM) which is required for mtDNA replication and transcription. It is known that the TFAM gene is overexpressed in many tumors and shows oncogene character. Aim of this study to determine the relationship between miR-200a-3p and TFAM in the pancreatic cancer cell line.

In our study, miR-200a-3p gene expression was increased by mimic transfection in Panc-1 and Miapaca-2 pancreatic cancer cell lines with low miR-200a-3p gene expression levels. It was shown that TFAM gene expression, which is highly expressed in pancreatic cancer cell lines, and TFAM decreased with the increase of miR-200a-3p gene expression. Furthermore, there was a negative correlation between increased miR-200a-3p gene expression and cell viability and a positive correlation between late apoptosis. As a result of mimic miR-200a-3p transfection, Panc-1 cell line proliferation was decreased.

These findings showed that TFAM is modulated by miR-200a-3p at the transcriptional levels, and TFAM could be one of the target genes for pancreatic cancer. Also, the miR-200a-3p expression level can be considered a target biomarker.

Keywords: Pancreatic cancer, micro-RNA, miR-200a-3p, TFAM

Construction of a new yeast adhiron library to screen for BCL6 binders

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Objective: Thermostable adhirons are derived from plant cystein protease inhibitors and have two loop regions that can be engineered to bind different targets. Here we aimed to construct a new yeast adhiron library and screen for binders against the BTB domain of the BCL6 transcription factor. BCL6 dimerizes through its BTB domains and acts as a transcriptional repressor. Impaired function of this gene could lead to diffuse large B cell lymphoma (DLBCL), the most frequent subtype of non-Hodgkin lymphomas (NHL). Thus, new BCL6-BTB targeting adhirons have therapeutic potential.

Materials-Methods: The nine amino acids of each loop region of a template were diversified using NNK containing primers in overlap extension PCR. Adhiron amplicons and a yeast expression vector (pYDS) were transformed together to *S. cerevisiae* yeast strain BJ5465 to allow gap repair mediated plasmid assembly. Transformants were selected in SD-Trp media and the growing yeast cells were pooled to form a yeast adhiron library. The serial dilutions of the transformants grown on SD-Trp agar plates were used to estimate library diversity. Individual yeast cells were checked for the presence of adhiron inserts by colony PCR. The uniqueness of inserts were determined by sequencing. The percentage of adhiron expressing yeast cells was assessed by flow cytometry using an anti-HA antibody after galactose induction for 48 hours.

Results: Colony PCR showed that each of the ten sampled colonies bears unique adhiron inserts demonstrating the success of transformation. According to the colony numbers on dilution plates the library diversity was estimated to be 1.3×10^8 . Flow cytometric assessment showed that 8.23% of the yeast cells expresses adhirons on cell surface.

Conclusion: This work is the first to construct a yeast surface display adhiron library. Adhirons targeting BCL6-BTB will be screened and could be used as therapeutics in the future.

Keywords: B-Lymphoma, BCL6, Adhiron, Yeast Surface Display

Biontech (BNT) Vaccine Induced Immune Neuropathy

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Background: A coronavirus disease is a life threatening respiratory disease. Therefore effective vaccine against coronavirus is crucial. However, some people experience adverse immunological responses to vaccines.

Case presentation: A 25-year-old man presented with complaints of numbness, tingling, burning and shooting pain in both hands and the feet. His complaints started one week after the BNT vaccine administration. On the neurological examination a distal sensory loss in both hands and feet was found. Electromyography (EMG) of four extremities was compatible with sensory motor neuropathy. His cerebrospinal fluid (CSF) revealed elevated protein. Other CSF components were in normal limits. With the diagnosis of vaccine induced acute immune axonal neuropathy, the patient was treated with intravenous immunoglobulin (IVIG). Four days after the IVIG treatment the patients' complaints disappeared. Three weeks later he came to the follow-up examination. On EMG examination his motor and sensory conduction values were partially improved. The second patient admitted with similar complaints which occurred five days after the BNT vaccine. He was 35 years old and his medical records were unremarkable. The neurological and EMG examinations of the extremities were compatible with sensory motor neuropathy. His CSF also revealed elevated protein. Other tests were in normal limits. With the diagnosis of vaccine induced immune neuropathy, the patient was treated with IVIG for five days. The patient's pain was relieved. On the follow-up EMG examination his sensory conduction values were improved gradually.

Discussion: Studies showed various immune mechanisms in vaccine induced autoimmune neuropathies including antigen mimicry, cytokine over expression, and activation of self-reactive T-cell clones. We presented these patients because the peripheral neuropathy after the BNT vaccine is a rare. Any adverse reaction to vaccines should be reported, and investigated to reduce vaccine hesitancy.

Keywords: Neuropathy, Peripheral neuropathy, Biontech vaccine, BNT, SARS-CoV-2

[PP-77]

The Modulatory Effect of Storage Time of Red Blood Cell Concentrates on The T Lymphocytes

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Objective: Investigation the effect of the storage time of red blood cell concentrates (RBC) on the T lymphocytes viability and proliferation ability.

Materials-Methods: Two units of whole blood (WB) were donated by volunteers. Also, a blood sample was taken from each volunteer before donation. RBCs were obtained from WBs and divided into five equal parts for the preparation of samples for 0, 7, 14, 21, 42 storage days (D0, D7, D14, D21, D42). Mononuclear cells (MNC) were isolated from RBCs and donor blood samples by the density gradient method (Ficoll 1077). Viability and proliferation analyses of T cell (CD3, CD4, CD8) were performed using PI and CFSE, respectively via a flow-cytometer. Additionally, the activation markers (CD25, CD69, CD154) were analysed in the PI-negative groups. All tests mentioned above were performed until D21. Tests about D42 could not be carried out due to their storage time didn't completed. Also, white blood cells (WBC) and MNCs were isolated from a volunteer by the density gradient method (Ficoll 1119 and 1077, respectively) for MTT tests. MTT analyses were carried out to investigate the effect of additive solutions (which exist in the RBCs), erythrocytes and phytohemagglutinin on the viability of these WBCs and MNCs.

Results: The most increased T cell viability was detected in blood samples taken before donation while the lowest level was seen in D0 samples. We observed that viability ascended during storage time compared to D0. The highest proliferation capability of T cells was detected in D14 samples. We determined that proliferation ability improved until D14, then it diminished to their lowest level in D21.

Conclusions: This study indicates that the viability of T cells in RBCs increases during storage time and their proliferation skills which are increased for a certain period then dramatically decreases.

Keywords: transfusion, transfusion related immune modulation, t cell, t cell poliferation, t cell viability

[PP-78]

Combination Of VLP-Based SARS-Cov-2 Vaccine With Omvs Of Neisseria Meningitidis Induces A Potent Immune Response In Mice

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Introduction

VLP vaccine against SARS-CoV-2 is based on the expression and assembly of four structural viral proteins known as spike (S), membrane glycoprotein (M), envelope (E), and nucleocapsid (N). Gram-negative bacteria release vesicular structures from their outer membrane, so called outer membrane vesicles (OMVs). These vesicles are the promising structures for vaccine development since OMVs carry many surface antigens that are identical to the bacterial surface. In this study, we mixed and encapsulated Delta Spike-expressing VLP with OMVs of 2 different strains of *N. Meningitidis* common in Turkey.

Materials and Methods

OMVs of serogroup B and serogroup W of *Neisseria meningitidis* has been isolated by ultracentrifugation. Their sizes and morphology were analyzed by qNano Gold system and atomic force microscopy (AFM). VLP and CpG ODN were mixed with OMVs or encapsulated into OMVs by lyophilization. Female C57BL/6 mice (6-8 weeks old) were intraperitoneally (i.p) injected with these formulations 2 weeks apart (on 0 and 14 days). Before the day of booster and 2 weeks post- booster injection, mice were bled and the sera were used to assess IgG responses against recS, Delta-RBD, recN, OMV B and OMV W.

Results and Discussion

Immunization resulted in high titers of total IgG, IgG1 and IgG2c responses against OMVs of both serogroups and all SARS CoV-2 viral antigens in all groups. Higher IgG2c over IgG1 titers indicated a strong Th1-biased SARS-CoV-2 and meningitis antibody response. Interestingly, lyophilized formulation had higher antibody titers compared to its counterpart.

Conclusion

These data demonstrate the immune potential of the VLP-OMV-based vaccine, suggesting that a combination vaccine containing bacterial and viral pathogens can be prepared.

Keywords: VLP, OMV, SARS-CoV-2, meningitis, vaccine

Determination of VLP vaccine immune potency in mice when combined with different adjuvants and complexed with liposomes

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Introduction

Virus like particle (VLP) vaccine expressing the four structural proteins of SARS-CoV-2 (Spike (S), Nucleocapsid (N), Membrane (M) and Envelope (E)) has been developed against the Covid-19. Liposomes enable high encapsulation efficiency for both proteins and nucleic acid based drugs, protecting the cargo from enzymatic cleavage while providing stability. In this study, we encapsulated Delta variant spike expressing VLP with K-type CpG ODN, poly(I:C) and cyclic dinucleotide 2'3'cGAMP (CDN) with different combinations.

Materials and Methods

Sterically Stabilized Cationic Liposomes (SSCL) are prepared by using the lipids DC-Chol, DOPE, PEG-PE. Liposomes are freeze-dried overnight after mixing them with VLP and adjuvants. VLP and adjuvants are encapsulated into liposomes by rehydration and subcutaneously injected to C57BL/6 mice. The sizes of liposomes were analyzed by Tunable Resistive Pulse Sensing. Booster injection is carried out 14 days after the primary injection and second booster injection is carried out 55 days after the booster injection. Mice were bled one day before the booster injections and Total IgG ELISAs were performed. 14 days after the last injection mice were bled again for Total IgG, IgG1 and IgG2c ELISAs.

Results and Discussion

SSCLs were similar in size and even enlarged after lyophilization. Total IgG titer values of every group increased over time, reaching to the highest points after 3rd injection. IgG2c titers of all groups, except (VLP)SSCL, were higher than their IgG1 titers. According to the IgG2c/IgG1 ratios, since they are bigger than 1, all groups with adjuvants elicited Th1 immune response against both viral proteins Spike and Delta RBD, whereas the group without adjuvants did not elicit Th1 dependent response.

Conclusion

In conclusion, these data suggest that the adjuvants CpG ODN, poly(I:C) and CDN favor Th1-biased T-cell responses.

Keywords: VLP, adjuvants, liposome

Cytoskeletal Dynamics in IL-1 β -induced Epithelial-Mesenchymal-Transition in Breast Cancer Cells

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IL-1 β is a common inflammatory mediator found in breast cancer microenvironment. It contributes to adhesion, invasion, and migration; therefore, epithelial-to-mesenchymal-transformation (EMT) is also modulated in cancer cells. Vimentin, vinculin, and CARMIL1-3 proteins are involved in the regulation of actin cytoskeleton, as critical elements of cell motility. Human breast cancer MCF-7 (epithelial phenotype) and MDA-MB-231 (mesenchymal phenotype) cell lines were seeded on Matrigel and fibronectin pre-coated surfaces in the presence or absence of IL-1 β . Expression, cellular localization of actin, vinculin, Vimentin and CARMIL1-3 proteins were quantified with the change in the morphology (surface area, polarization, focal adhesion patterns, membrane accumulation) of the cells by immunofluorescence. Gene expression levels for several markers were analyzed by qRT-PCR. Matrigel and fibronectin coating of surfaces had obvious effects on area and cytoskeletal protein expressions of MDA-MB-231 cells. Matrigel-coating enhanced focal particle patterns for all proteins. Fibronectin-coating further increased CARMIL1, and Vinculin while increased slightly CARMIL2-3 foci numbers compared to control. Although fibronectin-coating enhanced CARMIL1-3, and vinculin membrane accumulation compared to control, matrigel-coating had no influence. IL-1 β treatment induced mesenchymal morphology in MCF7 cells but not in MDA-MB-231 cells since these cells were already mesenchymal. Further, foci numbers of vinculin and CARMIL3 proteins were partly reduced with IL-1 β in uncoated setting, and vinculin was expanded while CARMIL3 was decreased in matrigel-coated setting in MB231 cells. Additionally, IL-1 β increased foci numbers of vinculin and CARMIL3 in both settings in MCF-7 cells. Even though membrane accumulation of vinculin was not affected by IL-1 β treatment in both cell lines, CARMIL3 was decreased in MB231 cells and increased in MCF7 cells. Gene expression levels of all proteins were increased with IL-1 β treatment in both MB231 and MCF7 cells. Our data shows that IL-1 β -induced EMT for breast cancer is associated with CARMIL proteins (especially CARMIL3) level and cellular distribution.

Keywords: IL1-B, cancer metastasis, adhesion

Monocyte/Macrophage Associated Immunomodulatory Function of CD44/CD24 Phenotype in Triple Negative Breast Cancer (TNBC) Subgroups on T Cell Responses

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Introduction: CD44+/CD24- breast cancer stem cell-like cells are the dominant subgroup of triple negative breast cancer(TNBC) but our limited understanding of their phenotypes and immunomodulatory effect on direct/indirect effect on T cell responses negatively affects achievement of treatment. This study aims to evaluate CD44/CD24 phenotype effect on monocyte/macrophage dependent immunofunctional differences.

Material-Method: MDA-MB-231,MDA-MB-468 basal-like breast cancer cell lines and THP-1 (monocytic), phorbol 12-myristate 13-acetate(PMA)-Induced THP-1(M0/macrophage), M2c-like THP-1(CD169+CD163+CD206dim) were co-cultured. CD44/CD24 subgroups from co-culture were purified with FACS. To measure their immunomodulatory character cells were co-cultured with PBMCs obtained from healthy donors and CD4+ or CD8+ T cell proliferation was tested with CFSE dilution by flow cytometry. CD80,CD86,PD-L1 and PD-L2 expression on cancer cells and immune cells were tested by flow cytometry for costimulation. CD163,CD169,CD206,CD14 were evaluated for macrophage polarization. Purified different CD44/CD24 cell subpopulation were plated for scratch assays and analyzed with ImageJ.

Results and Discussion: CD44+/CD24- subpopulation collected from M2c-like THP-1 cells/cancer cells co-culture condition, displayed less metastatic and more immunomodulatory capacity than THP-1 and PMA-Induced THP-1 conditions. In addition, CD163,CD169 vs CD14 expression were decreased on M2c-like THP-1 cells(CD44+/CD24- cancer cells primed). CD44+CD24+ population was decreased in THP-1 derived monocytic/macrophage-like cells coculture. In co-cultures between MDA-MB-468 cell lines and THP-1 derived monocytic/macrophage-like cells, especially CD44+CD24+ population was decreased. CD4+ or CD8+ T cells proliferation were affected to monocyte/macrophage-like cells primed CD44/CD24 sub-populations. In this study, CD44/CD24 phenotype in TNBC and their effects on monocytic/macrophage cell activation polarization were evaluated. In addition, the metastatic character and T cell responses of this interaction were detected, and the first preliminary data that could help immunotherapy approaches.

Keywords: CD4, CD8, T CELL, CD44, CD24 Monocyte, Macrophage

[PP-83]

Investigation the effect of anti-CD2 antibody coated wharton's jelly derived mesenchymal stem cells on stimulated T-lymphocytes in vitro

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Highly induced inflammation, like it was observed in the CoVid-19 infections, could have devastating effects on the tissue health and its function. In many studies, mesenchymal stem cells (MSC) have been shown to function appropriately to suppress this adverse interaction of T-lymphocytes with other cells by paracrine effect. However, the targeting of MSC to T-lymphocytes and the sublevel expression of cell adhesion receptors are considered among the important limitation of MSC-based therapies.

In this study, it was aimed to enhance the interaction of Wharton's Jelly (WJ)-MSC with T-lymphocytes by transferring the anti-CD2 (LFA2) antibody in a conjugated-complex with fatty acids to become a cell membrane element, and to investigate the effect of this interaction on phytohemagglutinin-stimulated T lymphocytes. After coating the anti-CD2 molecule to CD2⁺ MSC, the cell viability by WST, the cell counting by flow cytometer and cell-to-cell interactions by photomicrographs were evaluated.

The amount of fatty acid complex was first optimized due to its cytotoxicity effect in cell culture. Following the coating with conjugated-complex, the coating efficiency of WJ-MSC was found to be as 49,5% by flow cytometer. Under the microscope, the binding of T-lymphocytes with WJ-MSC was observed. The flow cytometer analysis also confirmed this interaction: the level of MSC bound with CD3⁺ T-lymphocytes was low, but the cell numbers were found to be higher in the anti-CD2 coated group, where the cell viability estimated by WST was higher.

By this experimental approach, the improvement of the effectiveness of WJ-MSC-T-lymphocyte cell interaction was observed. Thus, the immune modulatory properties of MSCs were improved significantly. The T-lymphocytes targeting of MSC via anti-CD2 in vitro showed that this approach might be applicable in the treatment of autoimmune diseases.

Keywords: CD2, Immunoregulatory, Paracrine, T lymphocytes

[PP-84]

Kinetic Characterization Of Various IL-1Ra Proteins Binding IL-1RI By Surface Plasmon Resonance (SPR)

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Objective: Familial Mediterranean Fever (FMF) And Behçet's Disease (BD) Are Autoinflammatory Disorders Causing Recurrent Inflammation Resulting In Irritation Of Various Body Parts. Both Diseases Have Common Characteristics In Clinical Outcomes And Are Thought To Be Genetically Inherited Autoinflammatory Disorders. Most Significant Common Feature Of These Diseases Is Upregulated IL-1 Expression. Recent Treatments Target IL-1 Or IL-1 Receptor Using Antibodies Or Antagonists (IL-1Ra) To Inhibit The IL-1 Signaling Pathway. In This Study, We Investigated The Affinity Characteristics Of Several Variant IL-1Ra Proteins And Compared Their Binding Activity Against IL-1RI Using Surface Plasmon Resonance (SPR).

Materials-Methods: IL-1Ra Proteins Were Expressed In E.Coli BL-21 Derivative Strains As C-Terminal His-Tagged Fusions And Purified Using Nickel Affinity Chromatography Followed By Size Exclusion Chromatography. SPR Experiments Were Performed On A Biacore200 Instrument. IL-1RI Was Immobilized Onto Flow Cell 2 Of A CM5 Sensorchip With ~40 RU By The Amine Coupling Method. Flow Cell 1 Of The Same Sensorchip Was Left Blank As A Reference. HBS-EP+ Was Used As The Running Buffer And Serial Dilutions Were Generated In This Solution. 5-Fold Dilutions Were Used For Multi-Cycle Kinetics (MCK) Analysis.

Results And Conclusions: On-Rate And Off-Rate Of In-House Produced IL-1Ra Were Compared Against The Originator Drug, Kineret. MCK Revealed Strong Molecular Interactions Between Anakinra (Active Substance Of Kineret) And IL-1RI. The Affinity Of Anakinra Was Calculated To Be 33.6-48.4 Pm While In-House Produced IL-1Ra Proteins And Anakinra Biosimilar Drugs On The Market Had Affinities Of 41.3-50.8 Pm. As A Preliminary Study, We Aimed To Determine And Compare The Affinity Of Several Variants Of IL-1Ra With That Of Anakinra Drug Substance. Using The SPR Technique, We Developed A Method To Investigate The Binding Properties Of IL-1RI Binding Proteins Which Will Be Used To Identify The Biosimilarity Of Different Protein Drugs That Target IL-1RI, In The Further Studies.

Keywords: Surface Plasmon Resonance, IL-1 Antagonist, Binding Affinity, Kinetic Analysis

Modulation of Pore-Forming Protein in Viral Infections

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Background. The Covid-19 outbreak caused by SARS-CoV-2 is still an important health problem that can be lethal. Hospitalized patients with severe Covid-19 were reported to present high levels of lactate dehydrogenase (LDH) and pro-inflammatory cytokine release. Inflammasomes are multimeric protein complexes regulating IL-1 β secretion through the activation of Caspase-1 and pyroptotic cell death through the cleavage of pro-Gasdermin D into its inactive form. Cleaved Gasdermin D oligomerizes and forms pores at the plasma membrane and triggers pyroptosis. Pathogens modulate host cell death and inflammasomes through different pathways to improve their survival. Gasdermin D can be inhibited by viral proteins such as nucleocapsids or by viral proteases. On the opposite, excessive activation of Gasdermin D can lead to the overactivation of the immune system and trigger cytokine storm.

Objectives. Based on the importance of cell death in the pathogenesis of Covid-19, in this study, we aimed to determine how different SARS-CoV-2 proteins modulate pyroptosis. For this purpose, we analyzed the main pyroptosis effector Gasdermin D.

Materials and Methods. To achieve our purpose, we cloned Gasdermin D into a mammalian expression vector and confirmed its expression in HEK293FT cells. Direct cleavage of Gasdermin D by viral proteins was tested in HEK293FT cells, an ectopic system that do not express any inflammasome components, by co-transfecting the cloned Gasdermin D with viral proteins. Activation of Gasdermin D via inflammasome complex was also assessed in THP-1 cells transduced by viral proteins. Gasdermin D cleavage was monitored by western blotting and pyroptosis was quantified by LDH assay.

Results and Conclusion. Several SARS-CoV-2 proteins were tested for Gasdermin D cleavage and each of them presented different patterns of pyroptosis activation both in LDH assay and western analyses. These results will contribute to the understanding of the molecular mechanism leading to Covid-19 and the development of effective drugs.

Keywords: Gasdermin D, Pyroptosis, LDH, Pores, SARS-CoV-2

[PP-91]

Characterization of a New Gasdermin Family Member's Role in Viral Infections

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Background. Gasdermins are a family of pore forming effector proteins that cause pyroptosis, which is a Gasdermin-dependent programmed inflammatory cell death. Gasdermin proteins have two distinct domains connected by a flexible linker: the N-terminal Gasdermin domain responsible for the pore forming at the plasma membrane and for the pyroptotic activity, and the C-terminal Gasdermin domain responsible for suppressing this activity when an activating signal is absent. Pyroptosis occurs in response to different stimuli activating cytosolic proteins called inflammasomes, and inflammatory caspases cleave Gasdermin D protein into its N-terminal effector domain releasing the inhibitory C-terminal domain. Oligomerization of the N-terminal domain leads to membrane rupture upon formation of large pores into it. Then, the intracellular pro-inflammatory cytokine interleukin (IL)-1 β is released through Gasdermin D pores. Recent research has linked SARS-CoV-2 infection to exacerbated inflammation and excessive pyroptosis. Blood samples from Covid-19 patients displayed high rate of pro-inflammatory cytokines and lactate dehydrogenase (LDH) release, markers of pyroptotic cell death.

Objectives. Considering the involvement of pyroptosis in Covid-19 pathogenesis, our aim was to investigate the ability of SARS-CoV-2 proteins to modulate Gasdermin B, a less studied member of the Gasdermin family.

Materials and Methods. For this purpose, Gasdermin B protein was cloned into a mammalian expression vector and its expression was verified in HEK293T cells. The ability of different SARS-CoV-2 proteins to induce Gasdermin B cleavage was determined by co-transfection of cloned Gasdermin B with different SARS-CoV-2 viral proteins and western blotting. Regulation of endogenous Gasdermin B's expression in response to viral protein transfection was also assessed.

Results and Conclusion. Our preliminary results suggest that Gasdermin B might be involved in SARS-CoV-2/innate immune system interaction. Characterization of Gasdermin B expression and activation in response to different SARS-CoV-2 proteins will provide valuable information on how this pathogen regulates pyroptosis.

Keywords: Virus, Infection, Pyroptosis, Gasdermin, Covid-19

[PP-92]

Among Individuals Carrying The HLA DR B1 *03:01 Allele, The Development Of Caga Positive Helicobacter Pylori Infection Is Significantly Higher

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Objective

CagA oncoprotein-containing strain of Helicobacter pylori is accused of causing gastric infection which also causes mucosal metaplasia. DR cell surface protein plays an important role for elimination of infectious agents. Aim of this study is to understand if there is a link between genetic polymorphisms of HLA DRB1 and H. pylori CagA IgA positive patients.

Materials and Methods

Patients with gastritis (n=174) were enrolled. CagA IgA, serum which shows active infection was analyzed by ELISA. HLA DRB1 allele frequency was analyzed by using PCR-SSO Method.

Results

According to our results, 134 patients are CagA Ig A negative while 40 patients are positive. There is no significant difference between gender and age distribution among 2 groups. HLA DR B1 *03:01 allele frequency is 0,092 (32/348) among all patients. However, 60 % of CagA IgA positive group carry *03:01 allele (n=14/80) while only 23% of CagA Ig A negative group carry DRB1*03:01 allele (n=18/268). Patients who carry HLA DRB1 *03:01 allele secreted CagA IgA immunoglobulin significantly higher frequency compared to non-*03:01 carriers (OR 0,33 (0,16-0,71) Maentel Haenzel Chi Square=8,56; p=0,003, 2-tailed).

Conclusion

HLA DR B1 *03:01 allele carrying DR protein on antigen-presenting cells might be less effective on surveillance and clearance of H. pylori with CagA oncoprotein which leads to higher rate of infection.

Keywords: HLA DRB1 *03:01, Helicobacter pylori, CagA IgA

[PP-93]

The Effect Of TRAIL-DR4/5 Signaling Activation On The Differentiation Of Primary Human Monocytes Into Macrophages

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TNF-related apoptosis-inducing ligand (TRAIL), a member of the Tumor Necrosis Factor Super Family (TNFSF), can bind to DR4 (TRAIL-R1) and DR5 (TRAIL-R2) death receptors in humans. Triggering of DR4/DR5 by TRAIL can induce apoptosis or cell survival. Although TRAIL and its receptors are expressed in many cell types in humans including immune cells, little is known about their immunomodulatory effects on immune cells.

In the present study, human primary monocytes from healthy donors were incubated with M-CSF and TRAIL during differentiation to macrophages for 7 days. Control groups were only cultured with M-CSF. Afterwards, both TRAIL treated and untreated macrophages were cultured for 1 day in the absence of TRAIL and then were either polarized into M1 macrophages which is a pro-inflammatory subtype, or kept as unpolarized, M0. The expression of M1 markers was analyzed by flow cytometry.

TRAIL stimulation during macrophage differentiation enhanced the production of M1 cell surface markers (CD86, HLA-DR alpha, and CD64) as well as M1 intracellular markers (CXCL10 and TNF alpha) in M0 macrophages at the protein level. Moreover, the production of the cell surface (CD86, HLA-DR alpha, CD64) and intracellular (CXCL10 and TNF alpha) M1 markers were elevated by TRAIL stimulation during macrophage differentiation in M1 macrophages.

In summary, our results show that TRAIL-DR4/5 signaling activation during macrophage differentiation drives monocytes to differentiate into M1-like macrophages. Our study sheds new light onto mechanisms of monocyte to macrophage differentiation by defining TRAIL as a new modulator.

Keywords: Monocytes, Macrophages, TRAIL, Death receptors

The impact of DR4/DR5 death receptors on macrophage polarization and cytotoxicity

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DR4 and DR5 death receptors are type I transmembrane proteins. Upon triggering of DR4/DR5 by TNF-related apoptosis-inducing ligand (TRAIL), cell death but also survival signaling cascades can be induced. Macrophages can be polarized into either pro-inflammatory/tumor-fighting M1 macrophages or anti-inflammatory/tumor-supportive M2 macrophages. The impact of DR4/DR5 receptors on macrophage polarization and cytotoxicity is not known and was investigated here.

Primary human monocyte-derived macrophages were pre-treated with either TRAIL or with DR4 or DR5-specific ligands and polarized into M1, M2a, or M2c phenotypes. The expression of M1/M2 markers in macrophages was analyzed by RNA-seq, qPCR, and flow cytometry. Furthermore, the cytotoxicity of the macrophages against AML tumor targets was measured by flow cytometry.

Our data demonstrated that TRAIL-induced DR4/DR5 signaling in macrophages increased the expression of M1 markers and decreased the expression of M2 markers. Furthermore, incubation with TRAIL significantly increased the cytotoxicity of M1 macrophages against AML tumor cells. To understand through which DR receptor TRAIL affects macrophage polarization, we treated macrophages with DR4 or DR5-specific ligands. While either DR4- or DR5-specific ligands led to an increased expression of M1 markers, the co-triggering of DR4 and DR5 together was required for the decreased expression of M2 markers in macrophages.

In conclusion, TRAIL supports the polarization of human macrophages into tumor-fighting M1 phenotype through both DR4 and DR5 receptors. Our data suggest that DR4/DR5 are novel drug targets to boost the anti-tumor activity of macrophages in the tumor microenvironment.

Keywords: DR4/DR5 death receptors, TRAIL, macrophage polarization, macrophage cytotoxicity

Polyherbal formulation *Kabasura Kudineer choornam* Polarizes Primary Human Macrophages into an M1 Phenotype

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Objective: *Kabasura kudineer choornam* (KKC) is a new polyherbal formulation containing 15 different ingredients. These ingredients have been separately shown to have anti-inflammatory, anti-pyretic and anti-bacterial properties. KKC has high binding affinity and interactions with SARS-CoV-2 spike protein shown in in-silico studies and exhibits good anti-viral properties against SARS-CoV-2 shown in a few clinical studies. However, the effect of KKC on macrophage polarization is not known. Here, we report a detailed analysis of the impact of KKC on the polarization of primary human monocyte-derived macrophages.

Materials-Methods: Human peripheral blood monocyte-derived macrophages (M0 macrophages), were either left untreated or pretreated with KKC extract at doses of 50 µg/mL, 100 µg/mL, 500 µg/mL, or 1.000 µg/mL for 2 h. Then they were polarized into M1 (LPS, IFN γ), M2a (IL-4), or M2c (IL-10) macrophages for 22 h. M1 and M2 surface markers were analyzed by flow cytometry. The M1 cytokine TNF was analyzed by ELISA.

Results: KKC extract treatment increased the expression of the M1 marker CD86 while decreasing the expression of M1 marker HLA-DR, M2a marker CD200R and M2c marker CD163 in M0 macrophages. In M2a and M2c macrophages, KKC extract decreased the expression of the M2a marker CD200R and the M2c marker CD163. Interestingly, after treatment with KKC extract, the phagocytic receptors CD64 and CD206 were upregulated in M0, M1, M2a, and M2c macrophages. Finally, KKC extract treatment enhanced the production of TNF in M0, M2a, and M2c macrophages.

Conclusion: KKC extract drives human macrophages into a virus-fighting M1 phenotype, with an upregulation of the phagocytic receptors CD64 and CD206. Thus, KKC may be a potential new supplement for modulation of macrophage polarization for the treatment of viral diseases, such as SARS-CoV-2.

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

Understanding Tissue-resident Macrophage Polarization in Basal-like Breast Cancer Metastasis

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Introduction and aim of the study: Macrophages present in solid tumor and metastatic site of different developmental origins are heterogeneous cell populations that greatly contribute to tumor progression. Both bone marrow-derived and tissue-resident macrophage populations contribute to tumor growth and metastasis. In past studies, the importance of tissue resident macrophages in the microenvironment in basal-like breast cancer metastasis in cancer prognosis has been found. In our study, the brain, lung and liver tissue from BALB/c mice were cultured w/wo bone marrow precursors and focused on the polarization and/or resident of these cells in the organs. At the same time, the role of factors secreted by triple negative breast cancer cells in macrophage polarization was investigated by tissue culture.

Material-Methods: Mice were sacrificed by perfusion method and circulating monocytes were eliminated. Lung, brain and liver tissues were obtained from BALB/c mice. Tissues were weighed and placed into DMEM/12 with compact or small pieces. They cultured with L929, 4T1 and EMT6 conditioned medium (L929 CM) w/wo bone marrow derived cells. Tissue infiltrating/resident macrophages were separated by using dispase and collagenase enzymes. Cells stained with CD11b, F4/80, CD11c, CD45 and evaluated by flow cytometry. Polarized macrophages from the culture were co-cultured with 4T1 and EMT6 cells, and the metastatic capacity of cancer cells was evaluated by scratch assay.

Results and Discussion: It is argued that the factors secreted by the primary tumor in breast cancer metastasis are responsible for metastatic organ formation. Although the effects of cancer-associated macrophages in this process are characterized, but there are no studies related the role of tissue-resident macrophages in this process, which are always present in organs. In this context, our study ongoing, and these part is one of our first preliminary work.

Keywords: Basal-like Breast cancer, tissue resident macrophages, metastasis

[PP-102]

Humoral Immunogenicity of VLP Vaccine Combined with K-type CpG, poly(I:C) and 2',3'- cGAMP adjuvants

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Introduction: Virus-like particle vaccine includes the virus's protein/s without genetic material. A VLP vaccine was developed and adjuvanted with Alum and K-type CpG ODN against SARS-CoV-2, currently being tested on Phase II Clinical trials. In addition to CpG ODN, a TLR9 agonist, herein we attempted to test the efficacy of other adjuvants and their combinations to reveal their possible clinical use. The additional adjuvants were poly(I:C), and a member of cyclic dinucleotides 2,3-cGAMP, (CDN hereafter), which are TLR3 and STING agonist respectively. In this study, we investigated the humoral immunity of C57BL/6 mice in response to the injection of a mixture of VLP with single, dual or triple adjuvant combinations.

Materials&Methods: 9 groups of male mice (C57BL/6, 6–8 weeks old) were intraperitoneally injected three times with mixture of VLP vaccine using different adjuvant combinations of CpG ODN, CDN, and poly(I:C). Blood was collected from the tail vein of mice two weeks after each injection. Antibody ELISA was used to determine humoral immunity against each combination of vaccines, and the plates were coated with in-house recombinant 6p-Spike, and commercial recombinant RBD.

Result&Discussion: Total IgG titers of mice in five different treatment groups were higher compared to VLP-alone group, and these showed a Th1-biased immune response whereas VLP-alone and VLP combined with CDN groups showed Th2-skewed immune response. Compared to the first injection, most groups' immunogenicity augmented significantly after the third injection. It was shown that while ii)CpG ODN, ii)poly(I:C), iii)CpG ODN+CDN, iv)CpG ODN+ poly(I:C), v)CpG ODN+CDN+poly(I:C) combinations worked synergistically, whereas CDN and poly(I:C) combinations demonstrated inhibitory effect.

Conclusion: In conclusion, these data suggest that individually CpG and poly(I:C) adjuvants and combinations of CpG and poly(I:C), CpG and CDN and triple adjuvant combination can be used in a vaccine as adjuvants to mount a Th1-skewed humoral immune response.

Keywords: SARS-CoV-2, VLP vaccine, CpG ODN

[PP-103]

Tissue resident/infiltrated macrophages isolation and their artificial expansion in 3D cells culture

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Introduction: Macrophages arise from bone marrow, yolk sac and fetal liver and tissue resident macrophages have lower plasticity than bone marrow-derived derivatives. In recent years, the role of tissue resident macrophages in several diseases has been emphasized. In recent years, the role of tissue resident macrophages in several diseases has been emphasized. There are limitations to obtaining and characterizing these cells. In this study, tissue resident macrophages isolation and their artificial expansion were tested.

Material-Methods: Lung, brain and liver tissues were obtained from BALB/c mice. Perfusion method was used to eliminate circulating monocytes. Tissues were weighed and placed into DMEM/12 with compact or small pieces. They cultured with L929 conditioned medium (L929 CM) w/wo bone marrow derived cells. Culture was maintained for 10 days and medium was refreshed every 4 days. While the spheroids were separated by pipetting, tissue infiltrating/resident macrophages were separated by using dispase and collagenase enzymes. After the tissues were kept at +4C overnight with dispase, collagenase was added the next day and incubated at 37C for 2 hours. Isolated cells from lung, brain, liver tissues and spheroids were layered over 1.077 g/ml and 1.119 g/ml Ficoll. Cells stained with CD11b, F4/80, CD11c, CD45 and evaluated by flow cytometry.

Results and Discussion: The phenotype and function of tissue-resident macrophages and bone marrow-derived macrophages are still unclear. Although there are many studies with the methods of obtaining from tissue, the efficiency is still a problem. In our study, with the model we used, the development, polarization and characterization of these cells were grown with a different culture setup. Macrophage polarization was evaluated by maintaining the interaction of bone marrow-derived cells and tissue. Preliminary data from this study will lead to experiments with these cells.

Keywords: macrophages, tissue resident macrophages, tissue culture, 3D culture

Immune Responses Of Children with Asthma During And After Sars-Cov-2 Infection

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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused coronavirus disease 2019 (COVID-19) pandemic and infected millions of people [1]. More than 6.5 millions of people have died from the infection worldwide [1]. Here, we aim to investigate the immune responses of children with asthma during after COVID-19 infection.

MATERIAL AND METHODS

Blood samples of children with (6 severe, 16 mild, 57 convalescent) and without asthma (19 severe, 18 mild) were collected. Serum cytokine levels and immunoglobulin responses of the patients were analyzed by cytokine bead assay (CBA) and ELISA methods, respectively.

RESULTS AND DISCUSSION

Immunoglobulin responses of the patients were similar in children except RBD IgM levels (Fig 1B). IgG, IgM and IgA levels showed a similar trend during 1-month of the follow-up. In asthmatic children with mild covid, IgA, IgG and IgM levels decreased after the 3rd month (Fig 1). In CBA assay, IL5, IL13, IL2, IL6, IL9, IL10, IFN γ , TNF α , IL17A, IL17F, IL4 and IL22 plasma cytokine levels were measured. In patients with asthma during severe covid, IL5 was insignificantly higher than children without asthma. On the other hand, IL10 was statistically higher in patients without asthma in mild infection, whereas IL17F was significantly lower in this group compared to children with asthma during mild infection. There was no difference between groups for other cytokines (Fig 2).

CONCLUSION

In conclusion, these results are compatible with literature data [1] and suggest that covid infection does not cause immunologically different responses in children with asthma compared to children without asthma.

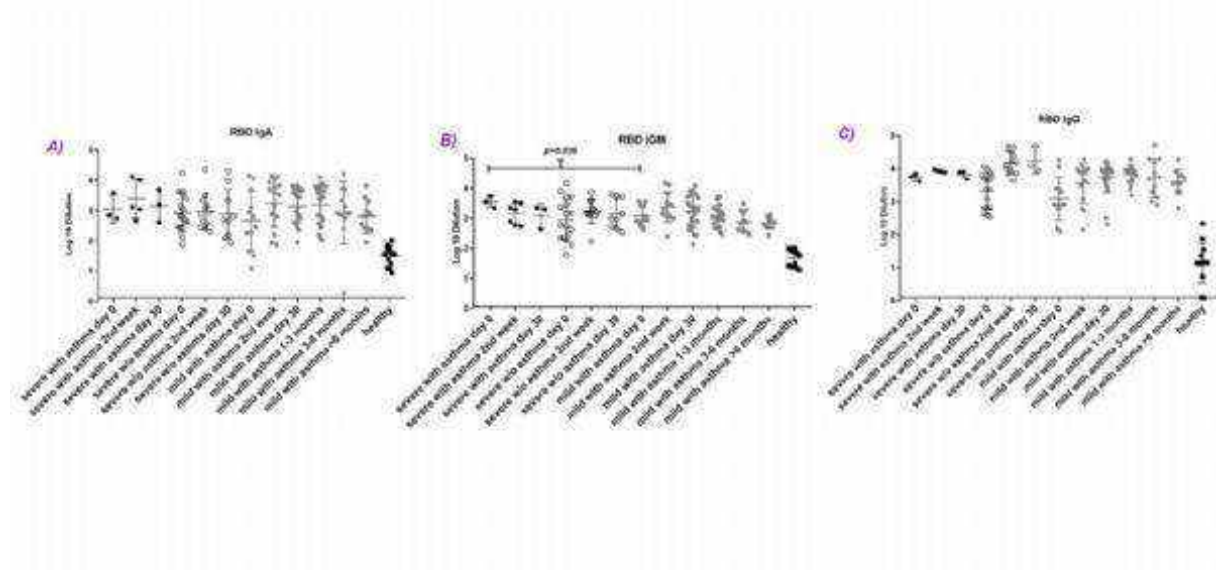
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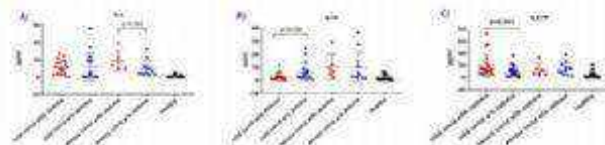
Keywords: asthma, children, cytokine, immunoglobulin, SARS-COV-2

Figure 1



A) IgA, B) IgM and C) IgG results against to receptor binding domain (RBD) of SARS CoV2 were determined in the plasma of the patients by ELISA

Figure 2



IL5, IL13, IL2, IL6, IL9, IL10, IFN γ , TNF α , IL17A, IL17F, IL4 and IL22 plasma cytokine levels of patients (day 0) with asthma (n=20 mild, n=6 severe) and without asthma (n=18 mild, n=12 severe) during severe and mild covid infection were measured by cytokine bead array method and compared with healthy controls (n=12). Only A) IL5 (p=0.083), B) IL10 (p=0.038) and C) IL17F (p=0.043) were different between patients and other cytokine levels were similar between groups (data are not shown).

Investigation Of Cellular And Humoral Immune Responses Induced By Sars-Cov-2 VLP Vaccine In Pre-Clinical And Clinical Studies

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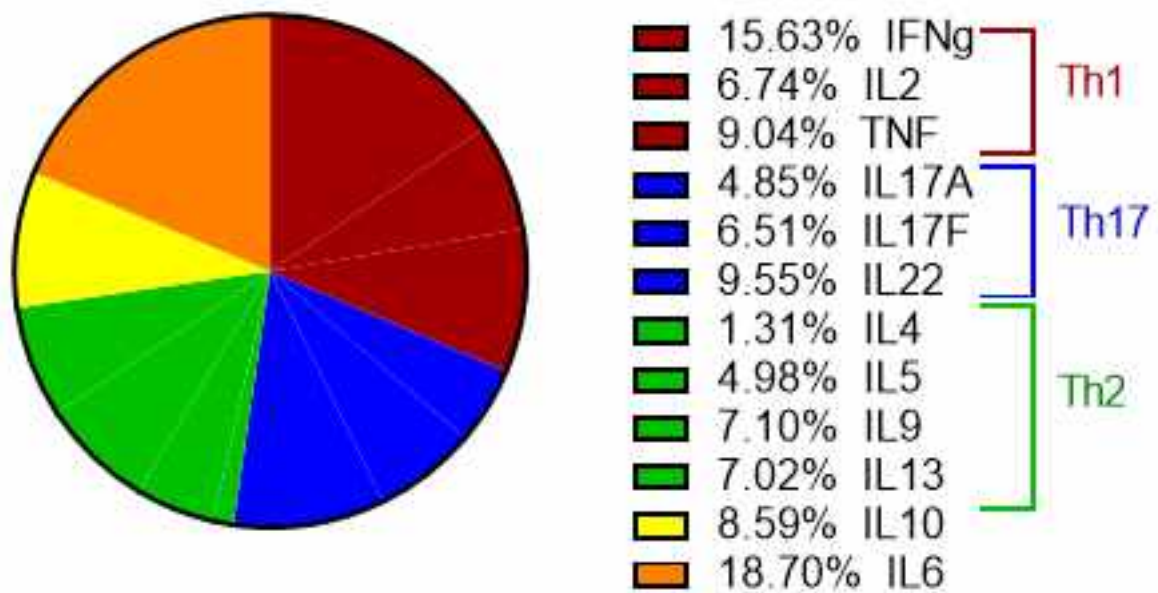
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Various vaccine studies are registered by WHO most of which target Spike protein or a certain region of Spike protein, such as receptor binding domain (RBD) for inducing immune reactions. Alternatively, the use of virus-like particle (VLP) technology for vaccine development broadens the magnitude of immune responses as the four main structural proteins of the virus, Spike, Nucleocapsid, Membrane and Envelope, are incorporated. Therefore, we developed a VLP vaccine against SARS-CoV-2 and adjuvanted it using Alum and K3 CpG ODN. Herein, we investigated the humoral and cellular immune responses induced by SARS-CoV-2 VLP vaccine in pre-clinical and clinical studies. The results from pre-clinical experiments conducted on vaccinated BALB/c mice indicated that VLP vaccine triggered effective antibody response against Spike, Nucleocapsid, Wild Type RBD, Alpha RBD and Delta RBD proteins as shown by ELISA. Additionally, it was proven that the diminishing antibody responses can be boosted and a prolonged immune response was achieved by a third dose of VLP vaccine. For the clinical studies, the humoral and cellular immune responses of Phase II clinical trial volunteers were analyzed by employing ELISA and CBA methods respectively. ELISA results demonstrated that the Phase II volunteers developed effective antibody titers against Spike, Nucleocapsid, Wild Type RBD, Alpha RBD and Delta RBD proteins. Analysis of the cellular immune responses by CBA showed that VLP vaccine induced a Th1-skewed immune reaction with coexistence of Th2-, Th17- and Treg related responses owing to its formulation with K3 CpG ODN and Alum. Taken together, our results indicated that SARS-CoV-2 VLP vaccine triggered effective cellular and humoral immune reactions against Spike and Nucleocapsid along with RBD variants which proved the cross-protective response achieved. Elicitation of multi-functional immune response and adoptability of the VLP technology to newly emerging variants makes VLP vaccine a promising candidate for the future booster injections.

Keywords: COVID-19, Virus-like Particle, Vaccine, K3 CpG ODN, Humoral immune response, Cellular immune response

Determination of Cellular Immune Responses



The percent distribution of Th1, Th2, Th17, T-reg and IL-6 based immune responses are presented in a pie chart.

Determination of The Tumor-Associated Antigens in Cutaneous Melanoma

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Introduction: Tumor-Associated Antigens (TAAs) are protein and glycoprotein-based substances that are derived from tumor cells. They are over-expressed in cancer cells compared to normal cells and also abundantly presented on the surface of cancer cells. Peptides of these TAAs bound to human leukocyte antigen (HLA) can be recognized by T cells initiating an anti-cancer immune response. Melanoma is one of the most aggressive cancers arising from the malignant transformation of melanocytes located in the basal layer of the epidermis. Skin cancers emerge as a serious public health problem in countries such as Turkey, which are exposed to intense sunlight during certain year seasons. In recent years, immunotherapies with cancer-specific adoptive cell transfer (ACT) have targeted intracellular tumor-associated antigens. In this study, we demonstrated the expression of TAAs identified previously in the literature in different stages and subgroups of cutaneous melanoma.

Methods: Immunohistochemical staining of tumor tissues of melanoma patients (n= 34) was performed on Ventana Benchmark XT automated instrument. Deparaffinization of embedded tissues was performed by keeping them at 50 C during the night. All tumor tissues were labeled with melanoma-associated markers such as TRP-1, TRP-2, NY-ESO-1, MAGE-A, MAGE-C1, Tyrosinase, HMB45, and MART-1. The expressions of these markers were determined by Alkaline Phosphatase Red Detection under the microscope.

Result: According to our results we observed that TRP-2 showed the highest expression in acral lentiginous, lentigo maligna, and nodular malignant melanomas while superficial spreading malignant melanoma had increased Tyrosinase expression. In addition to differential antigen expressions of melanoma-associated markers were also changed depending on the stages of the disease.

This study is being supported by The Scientific and Technological Research Council of Türkiye (TUBITAK), Project no: 218S910

Keywords: Tumor-associated antigens, Melanoma, Immunohistochemistry

TLR Ligand Loaded Exosome Mediated Immunotherapy of Established Mammary Tumor In Mice

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Tumor-derived exosomes (TEXs) could be harnessed as an immunotherapeutic cancer vaccine. These nanovesicles are inherently possesses rich tumor antigen reservoirs. Due to their undesirable features such as poor or limited immunogenicity as well as facilitation of cancer development via mediating communication between tumor cells, TEXs could be transformed into an effective immune adjuvant delivery system that initiates a strong humoral and cell-mediated tumor-specific immune response. Engineering TEXs to harbor immunostimulatory molecules still remains a challenge. Previously, we demonstrated that nucleic acid ligand encapsulated liposomes could trigger synergistic strong humoral, and cell mediated immune responses and provokes tumor regression to that of their standalone counterparts. In this study, we evaluated to immunogenicity of 4T1/Her2 cell-derived exosomes upon loading them with two potent immuno adjuvant, a TLR9 ligand, K-type CpG ODN and a TLR3 ligand, p(I:C). Engineered TEXs co-encapsulating both ligands displayed boosted immunostimulatory properties by activating antigen-specific primary and memory T cell responses. Furthermore, our exosome-based vaccine candidate elicited robust Th1-biased immunity as evidenced by elevated secretion of IgG2a and IFN γ . In a therapeutic cancer model, administration of 4T1 tumor derived exosomes loaded with CpG ODN and p(I:C) to animals regress tumor growth in 4T1 tumor-bearing mice. Taken together this work implicated that an exosome-based therapeutic vaccine promoted strong cellular and humoral anti-tumor immunity that is sufficient to reverse established tumors. This approach offers a personalized tumor therapy strategy that could be implemented in the clinic.

Keywords: Cancer Vaccine, TLR, Immune Response, Breast Tumor, Immunotherapy

The Effects Of Ovarian Induction On Mouse Uterine Dendritic Cells At Early Pregnancy

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Tarbiat Modares University

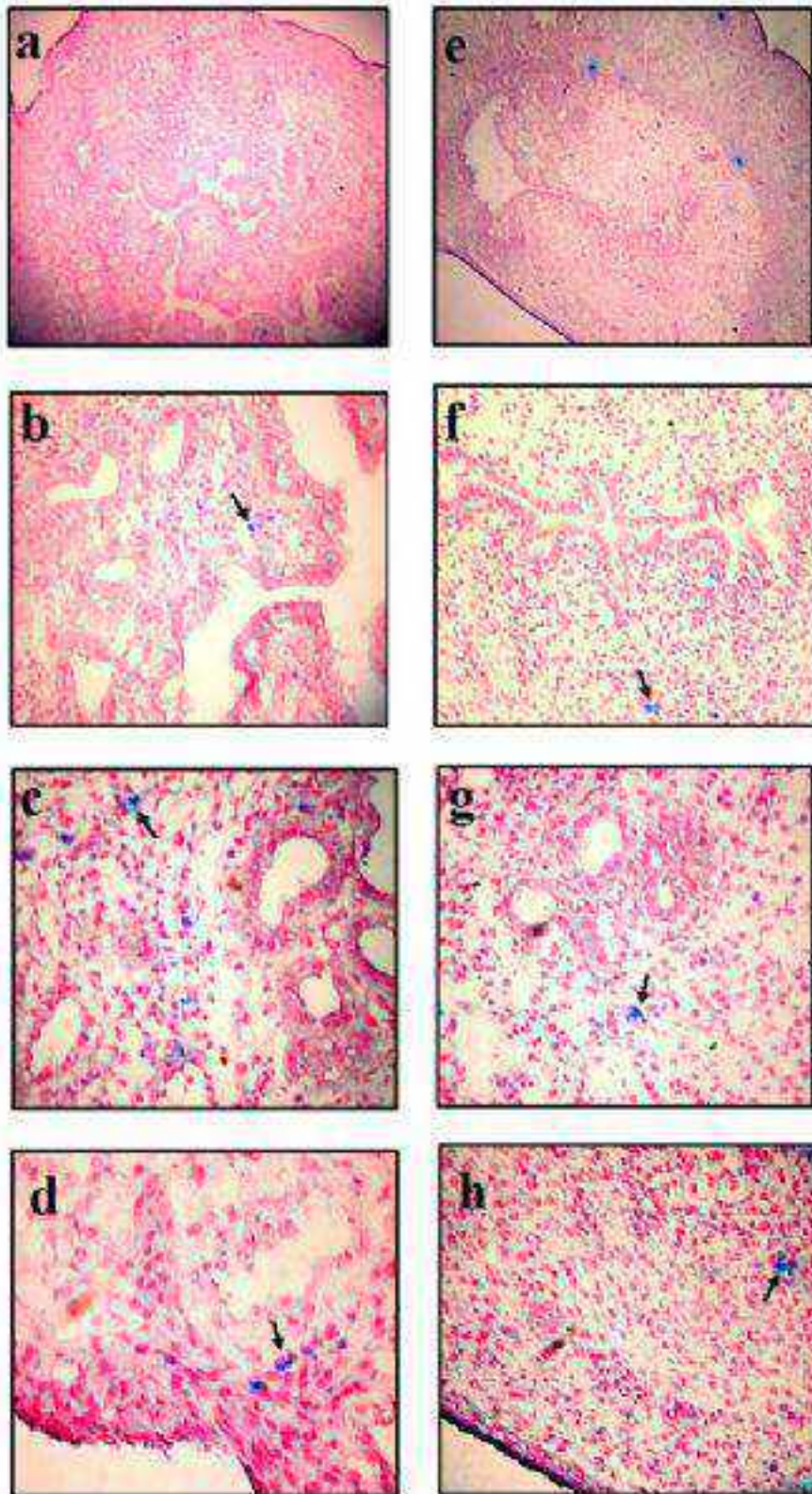
Introduction: ovarian hyper-stimulation is widely used in In vitro fertilization (IVF) clinics. The main purpose of this method is to stimulate folliculogenesis and increase the number of oocytes in one cycle. Following ovarian hyper-stimulation, hormonal secretion of the ovary, particularly estradiol and progesterone dramatically increases. Immune cells especially dendritic cells have receptors for the estradiol and progesterone and play an important role in appropriate implantation and successful pregnancy. Increase in estradiol and progesterone concentrations following ovarian stimulation can affect the recruitment and frequency of immune cells particularly dendritic cells.

Methods: To explore this issue, blood was collected from two groups of pregnant mice (with and without ovarian stimulation) on the seventh day of pregnancy. The amounts of estradiol and progesterone were measured in the sera. The frequency and localization of dendritic cells in spleen and decidua were also investigated by immunohistochemistry.

Results and Conclusion: The results of this study showed an increase of progesterone and estradiol concentrations and a decrease of frequency of dendritic cells in hyper-stimulated group compared to the control group. Considering the increase in progesterone and estrogen concentrations after ovarian induction and the presence of receptors for these hormones on dendritic cells, the changes in frequency of dendritic cells could be explained. Regarding the role of dendritic cells in embryo implantation and regulation of maternal immune response, it seems that their changes may decrease the rate of pregnancy success after IVF.

Keywords: Dendritic cell, Ovarian induction, Estradiol, Progesterone, pregnancy, IVF

Distribution of dendritic cells in the uterus of control (a-d) and hyper stimulated (e-h) pregnant mice



CTLA-4 Expression as a Candidate Marker for Immunotherapy in Neuroblastoma

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Objective: CTLA-4 is a T-cell suppressing protein that may play an important role in immune response to neuroblastoma. Expressions of CTLA-4 in neuroblastoma patients are correlated with survival. Our objective is to assess CTLA-4 levels in neuroblastoma patients. **Materials-Methods:** Our department is gathering and storing neuroblastoma patient samples from TPOG protocols. We used automatic immunohistostaining to determine CTLA-4 levels. Immunohistochemical expression of CTLA-4 were detected on sections of 94 neuroblastoma patients by the streptavidin-biotin method. Expression percentages were evaluated in mononuclear cells and statistically compared to clinical data.

Results: 52.1% of cases were male and 47.9% of cases were female. Mean age is 37.46±47.05 (1-192) months. According to the International Neuroblastoma Risk Group (INRG) classification; 7.4% of cases were low risk, 11.6% of cases were intermediate risk and 16.8% of cases were high risk group. 15.8% of cases were MYCN positive and 82.1% of cases were MYCN negative. CTLA-4 biomarker has a low percent positivity rate. 33% of the cases have more than %1 positive mononuclear cells within neuroblastoma tissue. 5.3 % cases have more than 5% expression. There was no correlation between MYCN amplification and CTLA-4 expression (p=0.232). Similarly, no correlation between risk group and disease stage and CTLA-4 positivity (p=0.845), (p=0.893).

Conclusions: In conclusion, CTLA-4 alone is not an effective prognostic biomarker in neuroblastoma. CTLA-4 positive cases might have benefit from anti CTLA-4 immunotherapy.

Keywords: Immunotherapy, CTLA-4, Neuroblastoma

[PP-112]

Amount of Non-Self RNA Is Matter to Regulate Nucleic Acid Sensor Retinoic Acid-Inducible Gene I (RIG-I) Activation

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The differentiation of cytosolic self vs non-self RNA is mainly dependent on detection of structural differences by nucleic acid sensors. They are crucial to detect RNA viruses and eliminate it through activation of interferon/ -stimulated genes (ISGs). In our previous studies, the infectivity rate of extracellular vesicles containing multiple virus particles is higher than infection with equivalent numbers of free viruses. Besides, innate immunity related genes decreased in vesicular form of infection. We evaluated whether this effect is due to viral nucleic acid amount presented in cytosol at the very beginning of the infection. We used several different viruses to infect different cell lines at low and high MOIs. We checked both viral RNA load and ifn- β expression via qPCR. The common trend was that while infection increases with higher MOIs, innate response was decreasing. We repeated our experiments using a mRNA analog, low molecular weight poly(I:C). When multiple non-self-viral nucleic acids enter the cytosol en masse, it results in suppression of RIG-I activation and decrease in interferon expression. Finally we demonstrated that this suppression of innate immune activation is in part due to the triggering of RIG-I protein degradation. Our study reveals a heretofore unknown cytosolic non-self RNA threshold above which viruses can suppress the activation of the innate immune system and replicate.

Keywords: Innate Immunity, RIG-I, Virus infection

Inflammatory Profile Of Human Brain Perivascular Fibroblasts Compared To Pericytes

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Objective: Brain pericytes and perivascular fibroblasts have recently been reported to contribute to disease pathogenesis in brain disorders including Alzheimer's disease, amyotrophic lateral sclerosis and multiple sclerosis. These two cell types are molecularly similar to each other and are located in close proximity in the perivascular area. Brain pericytes have recently been proposed to regulate immune homeostasis. However, immune-related functions of brain perivascular fibroblasts during neuroinflammation is not known. Here, we aim to investigate the immunological features of these two cell types comparatively.

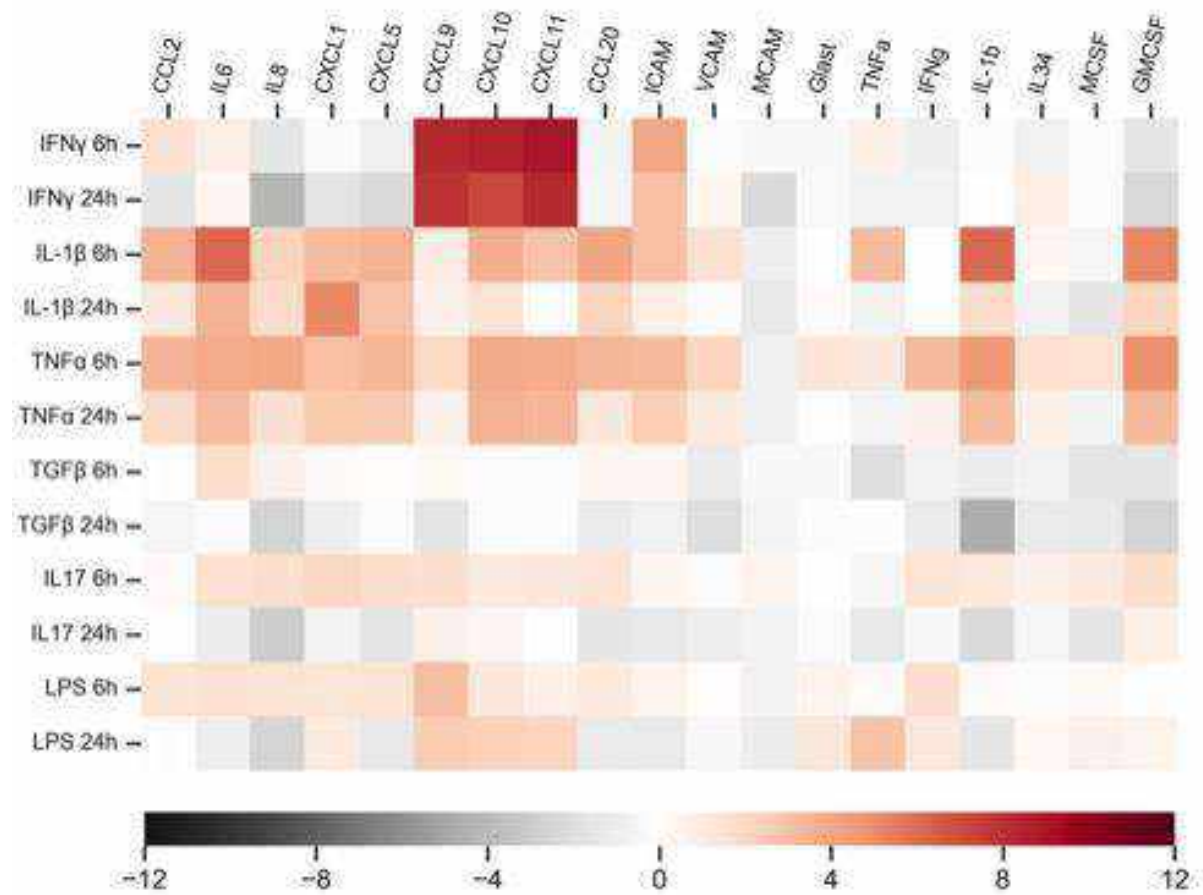
Materials-Methods: Commercially available pericyte and vascular adventitial fibroblasts obtained from human brain (Sciencell) were incubated with IL-1 β , IFN- γ , TNF- α , IL-17, LPS and TGF- β . Cytometric Bead Array (CBA), transwell migration assay and real-time PCR were used for analysis.

Results: Both pericytes and fibroblasts are responsive to inflammatory cytokines similarly at the transcriptional level. However, at the protein level pericytes stimulated with either IL-1 β , IFN- γ or TNF- α secrete most of the inflammatory molecules at higher amounts compared to fibroblasts. Specifically, CCL2, CXCL9, CXCL10, CXCL11 and M-CSF were expressed predominantly by pericytes. On the other hand, GM-CSF was secreted exclusively by fibroblasts. At the functional level, transwell assay performed with IFN- γ plus TNF- α stimulated cells showed that pericytes have a higher capacity to attract monocytes compared to fibroblasts. In addition, pericytes could also attract T lymphocytes although to a lesser extent compared to monocytes.

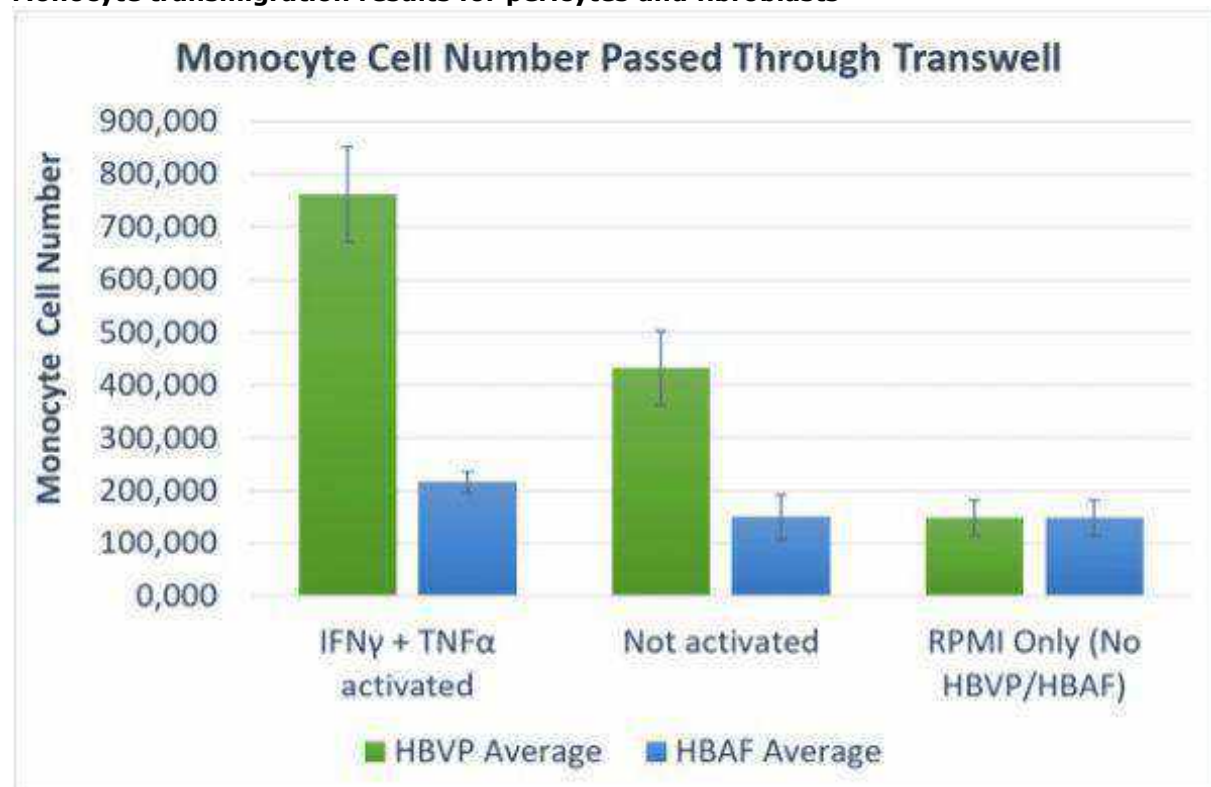
Conclusions: Our data shows for the first time that human brain perivascular fibroblasts are responsive to inflammatory cytokines. However, in general brain pericytes seem to be more immunologically active compared to fibroblasts, whereas there are differences in the expression of various cytokines between cell types. Future research will elucidate differential roles played by these two cell types during homeostasis and neuroinflammation.

Keywords: neuroinflammation, pericytes, fibroblasts, chemotaxis

Gene expression changes of cytokine treated pericytes



Monocyte transmigration results for pericytes and fibroblasts



Determination of Age-related Immunity Parameters in Sars-Cov-2 Vaccinations

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Virus-like particles (VLP) represent a highly effective vaccine platform with proven effective immunogenicity profiles. Their particulate nature enables display of antigens as repetitive units capable of cross-linking B cell receptors and hence trigger potent antigen specific humoral immunity. VLP based SARS-CoV-2 vaccine encodes the four structural proteins of the virus namely spike, membrane, envelope and nucleoprotein. However, despite their effectiveness in younger adults, vaccines may underperform in the elderly due to immune exhaustion and/or immune senescence. Therefore assessment of a vaccine's performance in different age groups is crucial for a complete understanding of its immunogenicity. Herein, the aim of the study is to assess the immunogenicity of a VLP based SARS-CoV-2 vaccine in the young-adult, adult and old mice. Our secondary goal is to strengthen SARS-CoV-2 specific vaccine induced immunity by modifying the adjuvant composition. To achieve these goals, we tried to optimize VLP and adjuvant (CpG and Alum) concentration in the vaccine formulation to elicit significantly elevated levels of antigen-specific IgG response in mice. We compared vaccine induced immune response of mice from different age groups. Our preliminary findings showed that VLP based SARS-CoV-2 vaccine induces significantly higher antigen specific IgG production than placebo control in all age groups. We also evaluated the efficacy of vaccine doses by cross-comparison between age groups. Since antigen specific antibody titers are cardinal measurement in vaccine efficacy, our study mainly focus on humoral response, i.e., IgG responses for readout. Along with the IgG titers, we also checked IgG subtypes to investigate Th1/Th2 dominance. Stemming from evidence in the context of vaccination history, we targeted Th1 dominated response since Th2 dominated response inducing viral vaccines associated with vaccine induced adverse effects(VIAE). Consistent with previous adjuvant research and vaccine trials, VLP based SARS-CoV-2 vaccine showed Th1-dominated immune response owing to CpG in the vaccine formulation.

Keywords: Vaccine, SARS-CoV-2, Virus-like particle, VLP, CpG, Adjuvant

[PP-115]

Effects Of Pyrimidines On Viability, Proliferation And Polarization In RAW 264.7 Macrophage Cell Line

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Introduction: Macrophages are the first line of defense against pathogens and their dysfunction (such as M1/M2phenotype and proliferation) can lead to serious health problems. Macrophage proliferation capacity, can be adversely affected by diseases such as cancer, infection or autoimmune diseases. Pyrimidines such as UMP,UDP,UTP are endogenous organic compounds.Previous studies showed that exogenously administered UMP,UDP,UTP stimulate the movement of monocytes and macrophages to the disease area and enhance phagocytic activities of macrophages.It was also previously demonstrated that pyrimidines enhance the growth, differentiation and proliferation of nerve cells in vitro.However, effects of UMP, UDP, UTP on the proliferation and polarization of macrophages have not yet been investigated.

Method: Raw264.7 mouse cell lines were transferred into tissue culture treated 6-well-plates and grown to 100%confluence. Upon reaching confluence, the cells in monolayer were vertically scratched using a tip. Uridine, UMP,UDP,UTP diluted in culture media was added to individual wells relevant doses ranging from 1,10,100µM. Images were captured every 24hours over a 3days period using an inverted light microscope to observe cellular migration(wound closure) and analysed by ImajeJ software. Proliferation effect of the Uridine,UMP,UDP,UTP on Raw 264.7 was determined using MTT assay. Cell viability was calculated as percentage change in comparison to the OD values obtained from untreated control cells.CD11b and CD206 expression were tested by flow cytometry.

Conclusion: According to MTT and scratch assay results, especially uridine and UTP were found to support proliferation at the end of 72 hours incubation. The percentage of CD11b+CD206+ cells were also increased in response to UTP and uridine stimulation.

Keywords: Uridine, RAW 264.7, macrophage, proliferation, MTT

[PP-116]

PRL2 (PTP4A2) Regulates Intracellular Magnesium Homeostasis And Activation-Induced Metabolic Fitness Of Naïve CD4 T Cells

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Metabolic programming plays a crucial role in T cell-mediated immunity for elimination of pathogens and tumors. Insufficient intake or alterations in magnesium (Mg²⁺) metabolism is thought to contribute to the inflammatory responses in human and mice. We have previously shown that PRL2 (Phosphatase Regenerating Liver-2) regulates intracellular Mg²⁺ levels in cancer cells. Yet, the precise role of Mg²⁺ and PRL2 in T cell metabolism relevant to maintenance and effector functions of T cells is largely unknown.

Here, by utilizing mice lacking PRL2, we examined the influence of this phosphatase on naïve T cell homeostasis and T cell receptor (TCR) mediated-signaling. Despite a reduction in splenic size, PRL2^{-/-} mice spontaneously accumulated T cells with an activated phenotype in spleen and lymph nodes. Surprisingly, PRL2^{-/-} mice had reduced serum interleukin-2 (IL-2) and interferon gamma (IFN- γ) levels. Upon in vitro TCR and CD28 co-stimulation, PRL2^{-/-} CD4 T cells exhibited a reduction in TCR-triggered induction of Notch1 and phosphorylation of mammalian target of rapamycin (mTOR) target ribosomal S6. This was accompanied by lessened IL2 and IFN- γ production and a reduction in cell cycle progression through S phase. A kinetic readout of T cell activation response revealed that PRL2 modulates cellular metabolic activation programs downstream of TCR ligation modulating intracellular and mitochondrial Mg²⁺ metabolism, mitochondrial ATP synthesis and aerobic glycolysis accompanied by lower concentrations of mitochondrial ROS production in activated T cells. Consistently, the clonal expansion of PRL2^{-/-} CD4 T cells were also reduced in response to lymphocytic choriomeningitis virus (LCMV) challenge and re-stimulation of these cells with LCMV peptides glycoprotein (gp61-80) produced less activated IFN- γ . In conclusion, PRL2 enhances activation-induced T cell metabolism downstream of TCR ligation, which is required for an appropriate T cell activation and cytokine responses. We propose PRL2 as an attractive therapeutic target in the treatment of T-cell mediated diseases.

Keywords: PRL2 phosphatase, magnesium, naïve T cell homeostasis, T cell activation, metabolism

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