MIMIC-II

1974

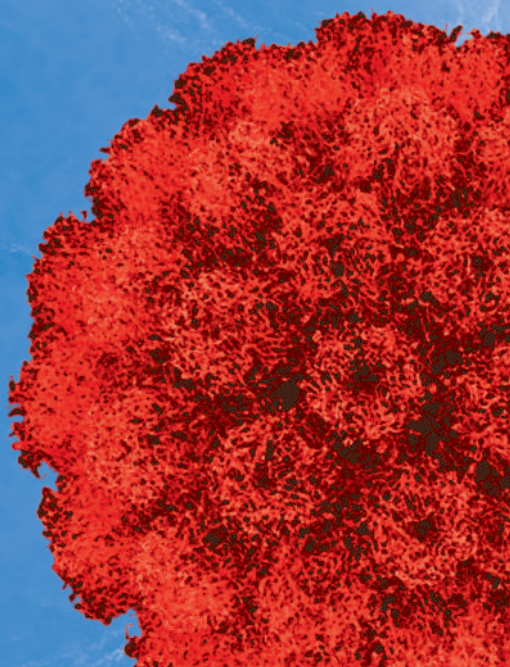
2nd International Molecular Immunology & Immunogenetics Congress (MIMIC-II)

April 27-30, 2014

Ramada Plaza Hotel

Antalya - TURKEY

PROGRAMME & ABSTRACT BOOK



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CONTENTS

WELCOME MESSAGE

Dear Colleagues and Friends,

We are honored to invite you ***to "2nd International Molecular Immunology & Immunogenetics Congress"*** (MIMIC-II) that will be held in Antalya, Turkey, from April 27 to 30, 2014 under the auspices of the Turkish Society of Immunology. The venue of the congress will be the "Ramada Plaza Hotel" in Antalya, a beautiful historical city and holiday resort on the shores of the Mediterranean Sea.

This congress will cover modern aspects of molecular and applied immunology as well as immunogenetics. Internationally renowned speakers will be giving talks during the congress. Since the main aim of MIMIC-II is to connect international scientists and immunologists to encourage new collaborations in the field of molecular immunology and immunogenetics, we invite scientists from all over the world to present their studies and to discuss the new findings and discoveries with other researchers.

Additionally, we are pleased to announce that the organizing committee has made special efforts to offer a large number of grants and awards covering registration, accommodation and part of travel expenses. Therefore, young researchers are particularly encouraged to apply for attending to the congress.

We are looking forward to seeing you in Antalya for this unique and stimulating event!

On behalf of the Organizing Committee,

Prof. H. Barbaros ORAL
Congress President

Prof. Gunnur DENIZ
President of TSI

SCIENTIFIC PROGRAMME

27 APRIL 2014, SUNDAY

13:00	Registration
15:00 - 15:30	Opening Ceremony "Colors of Anatolia" *Ferah Budak (TUR)*
OPENING CONFERENCE	
Chairs: H. Barbaros Oral (TUR) / Gunnur Deniz (TUR)	
15:30 - 16:30	NK Cells: New Approaches in the Therapy of Tumors and Leukemias *Lorenzo Moretta (ITA)*
16:30 - 16:45	Coffee Break
PLENARY TALK	
16:45 - 17:45	The Resting and the Restless Immunological Memory *Andreas Radbruch (GER)*
17:45 - 18:45	Immunology Taught by Viruses *Rolf Zinkernagel (SUI)*
18:45 - 19:30	Welcome Reception and Cocktail (Foyer)

28 APRIL 2014, MONDAY

NOVEL DISCOVERIES IN THE INNATE IMMUNE SYSTEM
Chairs: Sefik Sanal Alkan (SUI), Ihsan Gursel (TUR)

08:00 - 08:30	Tyrosine Phosphorylation Pathways in Myeloid Cells *Atilla Mocsai (HUN)*
08:30 - 09:00	Good and Bad Inflammation During Vaccination *Ken J. Ishii (JPN)*
09:00 - 09:30	Vaccine Adjuvant Activity of CpG Oligodeoxynucleotide/Cationic Peptide Nanorings *Mayda Gursel (TUR)*
09:30 - 09:45	Selected Oral Presentation Enhanced Sensitivity of Colon Tumor Cells to Natural Killer Cell Cytotoxicity After Mild Thermal Stress is Regulated Through HSF1 Mediated Expression of MICA *Baris Emre Dayanc (TUR)*
09:45 - 10:00	Selected Oral Presentation Enhanced Immunostimulatory Properties of Extracellular Vesicles Horboring Different TLR Ligands *Gozde Gucluler (TUR)*
10:00 - 10:30	Coffee Break

MICROBES AND MICROBIOMES: ROLES IN NON-INFECTIOUS DISEASES
Chairs: Nese Akis (TUR), Ishak Ozel Tekin (TUR)

10:30 - 11:00	Microbiota, Symbiosis and Dysbiosis *Sefik Sanal Alkan (SUI)*
11:00 - 11:30	Microbiome and Autoinflammatory Conditions *Ahmet Gul (TUR)*
11:30 - 12:00	Helicobacter-Induced Regulatory B Cells and their Role in Gastric Pathogenesis *Ayca Sayi Yazgan (TUR)*
12:00 - 12:15	Selected Oral Presentation Helicobacter Pylori Seropositivity Might Support the Immune Tolerance in Colorectal Cancer Cases *Ayse Basak Engin (TUR)*
12:15 - 12:30	Selected Oral Presentation Differential Activation of Immune Cells by Commensal versus Pathogen-derived Bacterial RNA *Mine Ozcan (TUR)*
12:30 - 14:00	Lunch Break

28 APRIL 2014, MONDAY

EMERGING ISSUES IN THE ADAPTIVE IMMUNE SYSTEM
Chairs: Cezmi Akdis (SUI), Vedat Bulut (TUR)

14:00 - 14:30	Human Tissue-Resident T Cells *René A. W. van Lier (NED)*
14:30 - 15:00	Immunity and Pathogenesis in Viral Infection *Daniel Pinschewer (SUI)*
15:00 - 15:30	Targeting Genes Important for the Immune System by Genome Editing *Batu Erman (TUR)*
15:30 - 15:45	Selected Oral Presentation Memory CD8+ T Cells Reside and Rest in Contact to IL-7 Producing Stroma Cells in Murine Bone Marrow *Ozen Sercan Alp (TUR)*
15:45 - 16:00	Selected Oral Presentation Magnified and Persistent Th1-biased Immunity Mediated by Exosome Vaccine *Tamer Kahraman (TUR)*
16:00 - 16:30	Coffee Break

REGULATORY CELLS OF IMMUNE SYSTEM
Chairs: Gulderen Yanikkaya Demirel (TUR), Mubeccel Akdis (SUI)

16:30 - 17:00	Pathogen-Induced Regulatory Cells Control Th1 and Th17 Cells that Mediate Autoimmunity *Kingston Mills (IRL)*
17:00 - 17:30	Molecular Mechanisms Driving Development of Lung Macrophages and Dendritic Cell Subsets *Manfred Kopf (SUI)*
17:30 - 18:00	Peripheral Tolerance Mechanisms *Mubeccel Akdis (SUI)*
18:00 - 18:15	Selected Oral Presentation Latency-Associated Protein Acr1 Impairs Dendritic Cell Maturation and Functionality: A Possible Mechanism of Immune Evasion by Mycobacterium Tuberculosis *Javed Naim Agrewala (IND)*
18:15 - 18:30	Selected Oral Presentation Effect of Breast Cancer Cell Lines on Myeloid Maturation and Differentiation *Gurcan Tunali (TUR)*
20:30	Poster Sessions

29 APRIL 2014, TUESDAY

TUMOR IMMUNOLOGY & MOLECULAR TARGETS *Chairs: Suhendan Ekmekcioglu (USA), Dicle Guc (TUR)*	
08:00 - 08:30	Adaptive Resistance: a Tumor Strategy to Evade Immune Attack *Gunes Esendagli (TUR)*
08:30 - 09:00	Organization and Clinical Impact of the Immune Microenvironment of Human Cancers *Catherine Sautes-Fridman (FRA)*
09:00 - 09:30	Monocyte Derived Suppressor Cells as Targets for Tumor Immunotherapy *Dennis Klinman (USA)*
09:30 - 09:45	Selected Oral Presentation Molecular Biomarkers of Inflammatory Signature and Its Role in Targeted Therapy Approaches in Melanoma *Suhendan Ekmekcioglu (USA)*
09:45 - 10:00	Selected Oral Presentation Identification by Microarray Analysis of Inmunological Molecular Markers Associated to Clinical Response in DC-vaccinated Melanoma Patients *Flavio Salazar Onfray (GER)*
10:00 - 10:30	Coffee Break
ALLERGY & TRANSPLANTATION IMMUNOLOGY *Chairs: Bilkay Basturk (TUR), Caner Susal (GER)*	
10:30 - 11:00	Modulation of Allergen-Specific Immune Responses: Novel Concepts, Tools and Platforms *Winfried Pickl (AUT)*
11:00 - 11:30	International Guidelines for Posttransplant Antibody Monitoring in Organ Transplantation *Caner Susal (GER)*
11:30 - 11:45	Ex Vivo Expanded and Genetically Modified Natural Killer Cells for Cancer Immunotherapy: from Process Optimization to Clinical Evaluation *Tolga Sutlu (TUR)*
11:45 - 12:00	Selected Oral Presentation The Tumor Suppressor p53 Interacts with PATZ1 Expressed During T Cell Development *Nazli Keskin (TUR)*
12:00 - 13:00	Lunch Break

29 APRIL 2014, TUESDAY

IMMUNODEFICIENCIES *Chairs: Yildiz Camcioglu (TUR), Sebnem Kilic (TUR)*	
13:00 – 13:30	Characteristics of Primary Immunodeficiencies in Turkey *Sebnem Kilic (TUR)*
13:30 - 14:00	The Different Faces of Chronic Granulomatous Disease *Isil Barlan (TUR)*
14:00 - 14:30	Severe Combined Immunodeficiencies: Clinical experience *Deniz Ayvaz (TUR)*
14:30 - 14:45	Coffee Break
DAY OF IMMUNOLOGY CONFERENCE: *Chairs: Lorenzo Moretta (ITA)*	
14:45 – 15:00	Introductory Speech by the President of EFIS
15:00 - 15:45	Resident Tissue Cells in Immune Tolerance and Chronicity *Cezmi Akdis (SUI)*
15:45 - 16:00	Selected Oral Presentation Acynetobacter Baumannii has got an Immune-evasion Mechanism *Handan Aksoy (TUR)*
16:00 - 16:15	Selected Oral Presentation FASL-844 T/C Polymorphism: A Biomarker of Good Prognosis of Breast Cancer in the Tunisian Population *Wijden Mahfoudh (TUN)*
16:15 - 16:30	Coffee Break
INFECTION TO IMMUNITY AND VACCINATION: NEW INSIGHTS & DEVELOPMENT *Chairs: Selim Badur (TUR), Cevayir Coban (JPN)*	
16:30 - 17:00	Immune Modulation by Helminth Parasites *Rick Maizels (GBR)*
17:00 - 17:30	Host-Pathogen Interactions in the Context of Malaria *Cevayir Coban (JPN)*
17:30 - 18:00	Longevity of Protection Provided by Vaccines: What is Known, What is Unknown? *Mustafa Bakir (TUR)*
18:00 - 18:15	Selected Oral Presentation Improving the Th1 Cellular Efficacy of the Lead Yersinia Pestis rF1-V Subunit Vaccine Using SA-4-1BBL as a Novel Adjuvant *Gunes Dinc (USA)*
18:15 - 18:30	Selected Oral Presentation Novel Formulations of c-di-GMP Enhance its Immunostimulatory Activity *Soner Yildiz (TUR)*
20:00	GALA DINNER (Meeting Hall - Carnavale-1)

30 APRIL 2014, WEDNESDAY

08:00 - 08:30	**RATIONAL DRUG USE** *Ihsan Gursel (TUR)*
NEUROIMMUNOLOGY *Chairs: Daniel Pinschewer (SUI), Guher Saruhan Direskeneli (TUR)*	
08:30 - 09:00	Inflammatory changes drive neurodegeneration in a model of Alzheimer's disease *Marina A Lynch (IRL)*
09:00 - 09:30	The Interplay Between Inflammation and Neural Stem Cells: Zebrafish Takes the Heat Up a Notch for Regenerative Therapies *Caghan Kizil (GER)*
09:30 - 10:00	Effect of T and B cells and the role of genetic predisposition during autoantibody production by Myasthenia gravis patients *Guher Saruhan Direskeneli (TUR)*
10:00 - 10:15	Selected Oral Presentation Iron and Iron Regulatory Proteins in Meninges and BMDM Cultures in NO-donor Induced Mouse Model of Migraine *Arzu Aral (TUR)*
10:15 - 10:30	Selected Oral Presentation Numerical Status of CD4+CD25+FoxP3+ and CD8+CD28-Regulatory T Cells in Multiple Sclerosis *Hassan Nikoueinejad (IRI)*
10:30 - 11:00	Coffee Break
AUTOINFLAMMATORY & AUTOIMMUNE DISEASES *Chairs: Akif Turna (TUR), Haner Direskeneli (TUR)*	
11:00 - 11:15	Selected Oral Presentation CD4 versus CD8 T Cell Lineage Fate Determinant PATZ1 Functionally Interacts with p53 *Emre Deniz (TUR)*
11:15 - 11:30	Selected Oral Presentation Role of CD40L Expressed by CD8+ T Cells in Cellular Immunity *Sibel Durlanik (GER)*
11:30 - 12:00	Biochemical Analysis of Lymphocyte Lineage Commitment Using the Locus-Specific Chromatin Immunoprecipitation Technology (iChIP and enChIP) *Hodaka Fujii (JPN)*
12:00 - 12:30	Genetics and Immuno-pathogenesis of Takayasu's Arteritis *Haner Direskeneli (TUR)*
12:30 - 13:00	Role of IL-1 Alpha and IL-Beta in Kawasaki Disease Vasculitis and Aneurysms. Lessons Learned from the Kawasaki Disease Mouse Model *Moshe Arditi (USA)*
13:00 - 13:30	**CLOSING CEREMONY**

LECTURES

NK CELLS: NEW APPROACHES IN THE THERAPY OF TUMORS AND LEUKEMIAS

Lorenzo Moretta

Istituto Giannina Gaslini, Genova

Natural Killer (NK) cells are important effectors of the innate immunity and play a relevant role in tumor surveillance and in defenses against viruses. Human NK cells recognize HLA-class I molecules through surface receptors (KIR and NKG2A) that inhibit NK cell function. Thus, they kill target cells that have lost (or underexpress) HLA-class I molecules as tumors or virus-infected cells. The "on" signal is mediated by an array of activating receptors and coreceptors that recognize ligands expressed primarily on tumors or virus-infected cells. NK cells have been exploited in the cure of high risk acute leukemias. Donor-derived "alloreactive" NK cells (i.e. that are not inhibited by the HLA-class I alleles of the patient) play a major role in the cure of both adult AML and pediatrics ALL patients. In these patients, alloreactive NK cells kill leukemia cells, thus preventing leukemia relapses, and patient's DC, thus preventing graft-versus-host responses. FACS analysis of KIRs expressed by NK cells allows to define the presence and size of alloreactive NK subset in potential haploidentical donors (i.e. parents and siblings) and to select the best one. We have recently shown that the expression of activating KIRs, in particular the (HLA-C2-specific) KIR2DS1, may also contribute to donor NK alloreactivity in patients expressing C2 alleles. Importantly, we established a clear correlation between the size of the alloreactive NK cell population and the clinical outcome. In this context, we have also shown that alloreactive NK cells are generated from donor's HSC and persist in patients for long time intervals.

Recently, haplo-HSCT has been further improved with the direct infusion, together with HSC, of donor-derived mature alloreactive NK cells and TCRγ/δ^+ T cells (obtained by depletion of TCRα/β^+ T cells and CD19$^+$ B cells). Both these cell types contribute to a prompt anti-leukemia effect together with an efficient defense against pathogens during the 6-8 week interval required for the generation of alloreactive NK cells from HSC. The preliminary results of this novel approach are particularly promising and further support the usefulness of NK cell-based immunotherapy of otherwise fatal leukemias.

Thus, the high survival rates of patients undergoing haploidentical HSC transplantation highlight an important new reality in the therapy of otherwise fatal leukemias.

THE RESTING AND THE RESTLESS IMMUNOLOGICAL MEMORY

Andreas Radbruch

Deutsches Rheuma-Forschungszentrum Berlin, a Leibniz Institute

Immunological memory is a hallmark of adaptive immunity. Plasma cells provide protective immunity, i.e. secreted pathogen-neutralizing antibodies, while memory B and T lymphocytes provide effective secondary immune responses to recurrent pathogens, they constitute a reactive memory. Despite its importance, however, little is known about how immunological memory is generated and maintained. Concepts range from persistent antigen and/or cytokines driving homeostatic proliferation and thus maintaining a population of longlived effector cells, to the discrete development of professional memory cells. For antibody-secreting plasma cells, we could demonstrate originally that longlived "memory" plasma cells are maintained in the bone marrow, to lesser extend also in secondary lymphoid organs and also in inflamed tissues, as long as they are inflamed. Plasmablasts, the precursors of plasma cells, migrate to the bone marrow, guided by the CX-chemokine ligand 12 (CXCL12), also called SDF-1α. In the bone marrow, they individually dock onto stromal cells which express CXCL12, and differentiate into memory plasma cells. These memory plasma cells rest in terms of proliferation and migration, while they secrete antibodies at high rates. CXCL12 expressing reticular stromal cells organize survival niches for memory plasma cells, by attracting accessory cells secreting essential survival factors for the plasma cells, like APRIL and IL-6. The most prominent accessory cells are eosinophilic granulocytes and megakaryocytes. One reticular stromal cell can only host one memory plasma cell, stromal cells thus define the "volume" of humoral memory as such. Once all niches are filled, new plasmablasts have to compete with preexisting memory plasma cells for niches.

In practical terms, the discovery of memory plasma cells impacts on the development of vaccines and even more on the development of therapies for chronic immune-mediated diseases. Memory plasma cells are resistant to todays state-of-the-art therapies. We have shown that autoreactive memory plasma cells can maintain such diseases on their own. The development of a cure for antibody-mediated diseases thus requires the development of new therapies eliminating (autoreactive) memory plasma cells.

Recenly, we found that memory T cells of the reactive memory are also maintained in the bone marrow as resting cells in terms of proliferation, transcription and mobility. Other groups have found that memory T cells specific for pathogens targeting the skin or mucosa also can rest in tissues like skin and lung, the tissue-resident memory T cells. These results challenge the current paradigm that memory cells are circulating throughout the body in quest of antigen, and are maintained by homeostatic proliferation. This may be true for memory B and T cells of the peripheral blood, which decay in numbers over time, and thus provide a kind of systemic shortterm memory for recently encountered pathogens. The circulating „restless" memory contains memory „stem cells", like IgM+ memory B cells and central memory T cells, but also effector memory cells, like IgG+ memory B cells and effector memory T cells, allowing a fast recapitulation of previous immune reactions. But apparently resting memory T helper cells of the bone marrow are essential to maintain a longterm memory for systemic pathogens and vaccines. In humans, Th memory to historic antigenic challenges, like measles, is maintained in the bone, not in the blood. As shown for mice, the precursors of bone marrow memory Th cells require CD49b (a2-integrin) and CD69 to get into the bone marrow. In the bone marrow, they dock onto stromal cells expressing IL-7 and collagen XI, the latter a ligand of CD49b. These stromal cells are different from the ones maintaining memory plasma cells. How the resting memory T cells of the bone marrow are reactivated, is still unclear. But their reactivation is required to mount efficient and longlasting secondary immune reactions.

TYROSINE KINASES IN NEUTROPHIL ACTIVATION AND MIGRATION

Attila Mócsai

Department of Physiology, Semmelweis University School of Medicine, Budapest, Hungary

Neutrophils are critical components of innate immunity but their improper activation may also lead to tissue damage during autoimmune inflammation. We have previously shown that integrin- and Fc-receptor-mediated neutrophil responses such as cell spreading, respiratory burst and degranulation required Src-family kinases, the Syk tyrosine kinase and the tyrosine phosphorylation-dependent phospholipase PLCγ2. Those studies prompted us to test the role of tyrosine phosphorylation pathways in in vivo inflammatory reactions and the accumulation of neutrophils at the site of inflammation. Src-family kinases, Syk and PLCγ2 were all required for autoantibody-induced inflammatory reactions such as the K/BxN serum-transfer arthritis in experimental mice. Given the role of tyrosine kinases in β_2 integrin-mediated neutrophil activation, we hypothesized that Src-family kinases, Syk and PLCγ2 are also required for β_2 integrin-mediated neutrophil migration. Surprisingly, neutrophil migration in a conventional Transwell assay did not require Src-family kinases, Syk or PLCγ2 even though it was strongly reduced by the genetic deficiency of the β_2 integrin-chain CD18. In addition, the Src-family kinase inhibitor dasatinib did not affect neutrophil migration through Transwell filters or a Matrigel matrix. In vivo competitive migration assays of neutrophils (in which wild type and knockout cells are allowed to migrate to the site of inflammation within the same animal) also revealed that Src-family kinases, Syk and PLCγ2 were not required for neutrophil migration in sterile peritonitis or autoantibody-induced arthritis models. On the other hand, tyrosine kinases were required for cytokine production by neutrophils and macrophages. Taken together, Src-family kinases, Syk and PLCγ2 are required for neutrophil activation and cytokine production but do not play any direct role in CD18-mediated neutrophil migration at the site of inflammation.

GOOD AND BAD INFLAMMATION DURING VACCINATION

Ken J Ishii[1,2]

[1] *Laboratory of Adjuvant Innovation, National Institute of Biomedical Innovation (NIBIO)*

[2] *Laboratory of Vaccine Science, Immunology Frontier Research Center (IFREC), Osaka University, Osaka Japan*

The word adjuvant has its origin from the Latin "adjuvare", meaning "to help". It is a general term for substances (factors) which are co-administered with a vaccine with the aim of increasing the effect (immunogenicity) of the vaccine. The research and development of adjuvants has a history of more than 80 years, and their actual mechanism was not immunologically understood for a long time, with a famous sarcastic remark "Immunologist's dirty little secret". Recent advance in Immunolgy; however, allowed the development of adjuvants through an innovative scientific approach, and there is fierce competition worldwide for the development of next-generation adjuvants. I would like to introduce and discuss about several adjuvants with their novel mechanisms, including a small compound as a potent DAMPs inducer to target certain innate immune mechanisms.

On the other hand, however, adjuvants range widely in terms of origin and mode of action, and they may be the cause or underlying cause of vaccine toxicity, especially immunotoxicity. I will present our recent work that common particulate adjuvants can cause local but sustained inflammation and allergic responses via novel innate immune mechanisms and cell death.

1. Kobiyama K et al Nonagonistic Dectin-1 ligand transforms CpG into a multitask nanoparticulate TLR9 agonist. ***Proc Natl Acad Sci U S A.*** 2014 111(8):3086-91.
2. Kuroda E, Coban C, Ishii KJ. Particulate adjuvant and innate immunity: past achievements, present findings, and future prospects. ***Int Rev Immunol***. 2013 32(2):209-20.
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4. Desmet CJ, Ishii KJ. Nucleic acid sensing at the interface between innate and adaptive immunity in vaccination. ***Nat Rev Immunol.*** 2012 12(7):479-91.
5. Marichal T et al. DNA released from dying host cells mediates aluminum adjuvant activity. ***Nat Med.*** 2011 17(8):996-1002.

VACCINE ADJUVANT ACTIVITY OF CPG OLIGODEOXYNUCLEOTIDE/CATIONIC PEPTIDE NANORINGS

Mayda Gursel

Middle East Technical University, Department of Biological Sciences, 06800, Ankara, Turkey

Structurally distinct classes of synthetic oligonucleotides (ODN) expressing CpG motifs differentially activate human immune cells. K-type ODN that progressed into human clinical trials as vaccine adjuvants/immunotherapeutic agents are strong activators of B cells and trigger plasmacytoid dendritic cells (pDCs) to differentiate and produce TNFa. In contrast, D-type ODN stimulate large amounts of IFNa secretion from pDCs. This activity depends on the ability of D-ODN to adopt nanometre sized G-quadruplex-based structures, complicating their manufacturing and hampering their progress into the clinic. In search of a D-ODN substitute, we attempted to multimerize K-ODN into stable nanostructures using cationic peptides. Here we show that a short ODN with a rigid secondary structure uniquely forms nuclease resistant nanorings following condensation with the HIV-derived peptide Tat(47-57). The nanorings enhanced cellular internalization, targeted the ODN to early endosomes and induced a robust IFNa response from human pDCs. Compared to the conventional K-type ODN, nanorings boosted Th-1 mediated immune responses in mice immunized with the inactivated Foot and Mouth Disease Virus vaccine and generated superior anti-tumor immunity when used as a therapeutic tumor vaccine adjuvant in C57BL/6 mice bearing ovalbumin (OVA)-expressing EG.7 thymoma tumors. These results suggest that the nanorings can act as D-ODN surrogates and may find a niche for further clinical applications.

MICROBIOTA, SYMBIOSIS AND DYSBIOSIS

Sefik S. Alkan

Basel, Switzerland

Microbes dominate as the most abundant life form on our planet, occupying almost every terrestrial, aquatic and biological ecosystem. We are used to view the immune system as a defense system developed to fight microorganisms. However, due to recent developments in the field of *metagenomics,* our understanding of entire living world has changed. We now think that entire life on earth depends on mutualistic partnerships *(symbiosis)* that improve their respective *evolutionary fitness.* The microbial communities that colonize living organisms are collectively called the *'microbiota'.* Together with microbiota, which sometimes carry 100 times more DNA than the host, all mammals can be considered as a "superorganism". Hosts learn to recognize, restrain and tolerate their commensals, which they encounter at birth, breastfeeding, etc. Both the microbiota and the host use tools shaped by millions of years of *co-evolution* to maintain a constant dialog and a mutualistic relationship. Under normal conditions, the commensal microbiota protects against colonization by pathogens. However, as with any relationship, things can go wrong. Small imbalances introduced by the host or microbes *(dysbiosis)* can disturb the dynamic equilibrium between host and commensals. In such circumstances, the microbiota can exert pathological effects that lead to disease such obesity, diabetes, inflammatory bowl diseases etc. Whereas there is much information on the composition of bacterial communities, much less is known about the viruses that colonize healthy people *(virobiota).* How these resident viruses (e.g. bacteriophages) can shape microbiota communities and influence host immunity is being investigated. Anatomical sites of a host other than the gut are also colonized by unique microbiota. The oral, vaginal and airway mucosa microbiota play more local roles such as tissue homeostasis.

In conclusion, armed with novel information about the host-microbiota interactions, translational attempts are now being made to treat obesity, diabetes, autoimmune diseases and even cancer, using microbes as a pill in combination with other agents.

References: *Seminars in Immunology 24 (2012); Seminars in Immunology 25 (2013); Nature Immunology 14 (2013); Nature immunology, 19 (2013); PLOS Biology (2013); PLOS Biology 12, (2014); Nature Rev Immunol. 13 (2013); Nature Rev Immunol. 14 (2014); PNAS 110 (2013); Nature Medicine 19 (2013); Science 339, (2013) ; Cell, 156, (2014).*

HELICOBACTER-INDUCED REGULATORY B CELLS AND THEIR ROLE IN GASTRIC PATHOGENESIS

Ayca Sayi Yazgan

Department of Molecular Biology & Genetics, Faculty of Science & Letters, Istanbul Technical University, Ayazaga Campus, Maslak, 34469, Istanbul, Turkey

Helicobacter infection is acquired during childhood and, despite triggering strong local and systemic immune responses, typically persist lifelong. Although a majority of infected individuals remain asymptomatic, ~20% develop one or more *Helicobacter*-associated severe gastric and duodenal disease manifestations; these include chronic active gastritis, ulcers, gastric B cell lymphoma, and, rarely, gastric adenocarcinoma. The knowledge related with protective mechanisms that operate in the majority of infected individuals not developing disease symptoms are limited. IL-10 producing regulatory B (Breg) cells were shown to suppress inflammatory responses in autoimmune pathologies and chronic inflammatory conditions. Recently, we have shown that B cells have the ability to negatively regulate adaptive immune responses to a bacterial pathogen, *Helicobacter*. Using mouse models of infection with *Helicobacter felis*, a close relative of the human gastrointestinal pathogen *H. pylori*, we found that B cells activated by *Helicobacter* TLR-2 ligands produce IL-10 and induce IL-10-producing CD4+CD25+ T regulatory-1 (Tr-1)-like cells *in vitro* and *in vivo*. Tr-1 conversion depends on TCR signaling and a direct T-/B-interaction through CD40/CD40L and CD80/CD28. B and Tr-1 cells cooperatively acquire suppressive activity *in vitro* and suppress excessive gastric *Helicobacter*-associated immunopathology *in vivo*. Characterization of IL-10 producing Regulatory B cells indicated that these cells express CD1d, CD5, CD21 and CD23 on their surface, do not secrete antibodies and their extracellular signal-regulated kinase (ERK) signaling pathway leads to IL-10 production. Our overall findings describe a novel regulatory B cell subset with an important immunomodulatory function during immune responses to persistent bacterial infections.

Our overall findings describe a novel regulatory B cell subset with an important immunomodulatory function during immune responses to persistent bacterial infections.

HUMAN CD8+ LUNG RESIDENT MEMORY T-CELLS

Pleun Hombrink[1], Berber Piet[2], Ronald A. Backer[1], Aldo Jongejan[4], Derk Amsen[1], René E. Jonkers[3], Perry D. Moerland[4], Klaas van Gisbergen[1] , René A. van Lier[1]

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The epithelial cells that line the respiratory tract are constantly exposed to the external environment. Respiratory viruses target epithelial cells as initial site of entry and replication. After infection, a specialized population of memory $CD8^+$ T-cells resides in the epithelium to maintain constant immune surveillance and protection against recurring respiratory infections[1]. Human lung tissue-resident T-cells (T_{RM}) express the integrin $\alpha_4\beta_7$ (CD103)[2] involved in interactions with epithelial cells by binding the ligand E-cadherin. We determined the transcriptional profile of T_{RM} retrieved from human lung resection samples. A comprehensive set of transcription factors was identified that characterizes lung resident T_{RM}. To this set belongs Hobit (ZNF683), a transcription factor highly homologous to Blimp-1[3]. Moreover we found that the expression of both the NOTCH1 receptor and a large number of NOTCH1 target genes is high in lung derived T-cells and even more pronounced in those that express CD103. The relevance of NOTCH1 for the formation of T_{RM} could be confirmed in an influenze model in NOTCH1/2 deficient mice. Our data illustrate the adaptation of lung resident T-cells to the requirements of the respiratory epithelial environment. Defining the molecular imprinting of these cells is important for rational vaccine design and may help to improve the properties of T-cells for adoptive cellular therapy.

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IMMUNITY AND PATHOGENESIS IN VIRAL INFECTION

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Virus-host relationship is multifaceted and allows for observations on governing principles in the immune system. In the last decade it has become widely accepted that pathogen-associated molecular patterns decisively influence antiviral immune responses. Conversely, the contribution of endogenous signals of tissue damage, also known as "damage-associated molecular patterns" or "alarmins" remains ill-defined. We observed that interleukin-33 (IL-33), an alarmin released from necrotic cells, is necessary for potent CD8+ T cell (CTL) responses to several replicating, prototypic RNA and DNA viruses in mice. IL-33 signaled through its receptor on activated CTLs, enhanced clonal expansion in a CTL-intrinsic fashion, determined plurifunctional effector cell differentiation and was necessary for virus control. Moreover, recombinant IL-33 augmented vaccine-induced CTL responses. Radio-resistant cells of the splenic T cell zone produced IL-33, and efficient CTL responses required IL-33 from radio-resistant cells but not from hematopoietic cells. These observations suggest that alarmin release by radio-resistant cells is key for the orchestration of protective antiviral CTL responses.

GENOME ENGINEERING OF THE IL7R GENE WITH TALEN VE CRISPR/CAS9

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T lymphocytes survive by persistent signaling from cytokine receptors on their cell surface. Interleukin 7 receptor (IL7R) mediates cell survival upon signaling by the soluble cytokine IL7. The expression of the gene encoding IL7R is tightly controlled during differentiation in the thymus and during the immune response in the peripheral immune system. Inappropriate signaling as a result of dominant mutations in IL7R gene result in acute T lymphoblastic leukemia. We aimed to identify the mechanisms controlling IL7R gene expression. We have identified cis-regulatory regions in the IL7R gene locus and the transcription factors that associate with these sequences such as Gfi1, Glucocorticoid receptor, Notch and NFkB. We mutated important cis-regulatory regions in the IL7R gene locus using TALEN and Crispr/Cas9 genome editing tools. TALENs are artificial proteins derived from "transcription activator like-TALE" proteins of the plant pathogenic bacteria belonging to the *Xanthomonas* genus. Crispr/Cas9 proteins are derived from the innate immune system of bacteria. We have used both genome editing tools to generate double stranded breaks in the genome of tissue culture cell lines specifically in the transcription factor binding sites of the cis-regulatory regions of the IL7R gene. Double stranded breaks result in inaccurate repair by non-homologous end joining (NHEJ) and insertions and deletions. We show that site directed mutagenesis of the genomic IL7R gene locus results in decreased IL7R expression.

PATHOGEN-INDUCED REGULATORY CELLS CONTROL TH1 AND TH17 CELLS THAT MEDIATE AUTOIMMUNITY

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Effector CD4 T cells that secrete IFN-γ (Th1 cells) or IL-17 (Th17 cells) mediate protective immunity to infection by promoting recruitment and activation of macrophages and neutrophils respectively. Th1 and Th17 cells also play a pathogenic role in many autoimmune diseases. Regulatory T (Treg) cells can suppress Th1 and Th17 responses and thereby prevent autoimmunity and infection-induced immunopathology. However, Treg cells can also be exploited by pathogens to subvert host protective immunity (1). Stimulation of dendritic cells by pathogen-derived molecules promotes maturation and T-cell promoting cytokines (2). We have shown that TLR and NLR agonists induce innate IL-1 and IL-18 which synergize with IL-23 to promote activation of Th17 cells and innate IL-17 production by γδ T cells (3). The induction and function of Th1 and Th17 cells is regulated by cytokines secreted by the other major subtypes of T cells, especially IL-10 and TGF-β production by Treg cells but also by regulatory cells of the innate immune system. The induction of adaptive Treg cells is stimulated by retinoic acid, TGF-β and IL-10 in response to certain virulence factors from pathogens, such as helminth parasites that have evolved sophisticated mechanisms to subvert host protective immunity (4). Pathogens and pathogen-derived molecules can also promote activation of alternatively activated (type 2) macrophages, type 2 innate lymphoid cells (ILC2) and tolerogenic dendritic cells that can suppress Th1 or Th17 cells, either directly or through the induction of Treg cells. We have identified approaches for activation of anti-inflammatory cytokines and regulatory innate immune cells and for selective induction of Treg cells, without Th1 or Th17 cells. These approaches been effective in attenuating inflammatory disease in preclinical models of autoimmunity.

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PPARγ PROVIDES A SECOND ESSENTIAL SIGNAL SUBSEQUENT TO GM-CSF FOR NEONATAL TERMINAL DIFFERENTIATION OF ALVEOLAR MACROPHAGES

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Tissue-resident macrophages comprise a diverse group of professional phagocytes found in most organs. A unifying characteristic of these evolutionary conserved cell types is their important function in tissue homeostasis. It has been recently established that the majority of resident tissue macrophages originate from common embryonic progenitors that are generated during early haematopoiesis in the yolk sac and fetal liver. The development of diverse tissue macrophages from common embryonic progenitors indicates the requirement of local tissue specific extrinsic signal that induce cell intrinsic signals driving the differentiation of the progenitors to specialized effector cells with distinct gene expression profile in a tissue-specific manner. While the cell intrinsic signals remain poorly defined, the extrinsic signals for development of some tissue macrophages are beginning to emerge. Here we identify peroxisomeproliferator-activated receptor-γ (PPARγ) as the crucial transcription factor imprinting alveolar macrophages (AM) identity and regulating signature gene expression required for perinatal development of AM. Development of AM was largely abrogated in CD11c-Cre/*Pparg*$^{fl/fl}$ and vav-Cre/*Pparg*$^{fl/fl}$ mice due to a perinatal block the stage of a fetal monocyte-derived AM progenitor. By contrast, PPARγ was dispensable for development of macrophages located in heart, kidney, lamina propria, and white adipose tissue and for conventional DCs. Transcriptome analysis of pre-AM from 2 days old wild-type and CD11c-Cre/Ppargfl/fl mice unraveled that PPARγ confers a unique transcriptional signature program including several transcription factors associated with AM differentiation and AM function (i.e. lipid transport and metabolism). Intriguingly, fetal monocyte-derived AM progenitors expressed high levels of PPARγ, which is dependent on local expression of GM-CSF in the embryonic lung. Overall, we provide evidence for a lung-specific role of GM-CSF inducing perinatal AM development at least partially by induction of PPARγ in fetal monocytes.

TOLERANCE MECHANISMS TO ALLERGENS

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B cell play a unique role in the shaping the immune system through production of antibodies. Besides this classical function, B cells can regulate immune responses through the production of cytokines. IL-10-producing regulatory B cells can negatively regulate inflammatory immune responses and have been linked with protection against various autoimmune diseases in murine models. A functional role from human regulatory B cells was supported by reports of exacerbation of ulcerative colitis and development of psoriasis after B cell depletion therapy with the anti-CD20 mAb rituximab. Furthermore, human IL-10-producing CD24hiCD38hi B cells were functionally impaired in systemic lupus erythematosus patients, suggesting a role for regulatory B cell in controlling human autoimmune disease. The aim of this study was to characterize human IL-10-producing regulatory B cells and study their function in relation to immune tolerance induction towards allergens.

We identified a subset of human B cells that produces large amounts of IL-10 in response to the TLR9 ligand CpG2006. We termed this B cell subset, which expresses high levels of CD25, CD71 and low levels CD73, inducible B regulatory 1 (Br1) cells, in analogy with inducible TR1 cells. Br1 cells could efficiently suppress antigen-specific CD4+ T cell proliferation in an IL-10-dependent manner. We could demonstrated this in an *in vitro* system in which PPD-stimulated PBMC were co-culture with autologous Br1 cells in the presence or absence of a neutralizing anti-IL-10 receptor mAb. Br1 cells suppressed 50% of PPD-induced CD4+ T cell proliferation at a ratio of 1 Br1 cell to 25 PBMC.

We hypothesized that if a B cell plays an anti-inflammatory role, the antibody isotype produced by the same B cell when it differentiates into a plasma cell should support the notion of being anti-inflammatory. Purified naïve (CD27-) Br1 cells selectively upregulated the production of anti-inflammatory IgG4 antibodies, while other immunoglobulin isotypes (IgG1, IgA) were produced in equal amounts by IL-10- and IL-10+ (Br1) cells. These findings demonstrate that Br1 cells have the propensity to switch to IgG4-producing plasma cells.

A shift towards IL-10-producing allergen-specific Tr1 cells during tolerance induction has been reported in beekeepers. Furthermore, healthy IL-10-producing Tr1 cells are the dominant allergen-specific Th cell subset in healthy individuals while IL-4-producing Th2 cells has a high frequency in allergic individuals. [7] In the current study we sought to identify a potential role for Br1 cells in the maintenance and induction of immune tolerance towards allergens. As a human *in vivo* model we used highly exposed beevenom tolerant beekeepers and beevenom allergic patients before and 110 days after ultra rush beevenom-specific immunotherapy. B cells specific for the major beevenom allergen phospholipase A2 (PLA) that were isolated from peripheral blood of tolerant beekeepers expressed significantly higher levels of mature IgG4 mRNA transcripts and IL-10 when compared to non-PLA-specific B cells. In order to quantify the frequency of PLA-specific IL-10-producing B cells, total B cells were purified from peripheral blood of beekeepers and beevenom allergic patients before and after immunotherapy. Purified B cells were stimulated for 3 days with CpG2006, and the frequency of IL-10-producing PLA-specific B cells was assessed by flow cytometry. The frequency of IL-10-producing B cells in beekeepers was on average 1.8-fold higher in PLA-specific B cells than in non-PLA-specific B cells. Interestingly, the frequency of IL-10-producing B cells from allergic patients before immunotherapy did not differ between PLA-specific and non-PLA-specific B cells. This frequency was however significantly increased after immunotherapy (mean increase 3-fold) to levels comparable to those observed in tolerant beekeepers. Furthermore serum IgE and IgG4 specific for PLA was measured from beekeepers and patients before and after immunotherapy. Allergic subjects after immunotherapy had a >100-fold lower PLA-specific IgE/IgG4 ratio when compared to patients who did not receive immunotherapy, while this ratio was >1000-fold lower in beekeepers compared to allergic individuals.

Taken together, our data strongly supports a role for IL-10-producing Br1 cells in peripheral tolerance induction mediated by secretion of IL-10 and production of IgG4. Furthermore B cell derived IL-10 may suppress differentiation and maturation of monocytes (unpublished data).

ADAPTIVE RESISTANCE: A TUMOR STRATEGY TO EVADE IMMUNE ATTACK

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Keywords: Cancer, PD-1, ICOS, costimulation, immune evasion

The straightforward perspective that tumor cells are immunosuppressive has been contradicted by the expression of potent costimulatory molecules on certain cancer cells. Indeed, the stimulatory support offered by certain cancer cells such as myeloid leukemia or basal-like breast cancer cells can provoke helper T cell responses. Unfavorably, this interaction can lead these malignant cells to acquire an immune suppressive capacity.

Strong immune reactions are required for the elimination of tumor cells.[1,2] Tumor cells found in the circulation are considered to be more susceptible to immune attack; the likelihood of these cells to come across with immune cells is enhanced, and they are devoid of a protective (immunosuppressive) microenvironment.[1] On the other hand, anti-tumor immunity does not always correlate with reduction in tumor growth and increase in patient survival.[1,2] Moreover, immune stimulatory interventions **have been shown to enhance the ability of immune cells to eradicate some, but not all, tumor cells.**[2] Essentially, reduction in immune-provoking signals derived from the tumor can diminish the effector phase of immune responses. The dogma in tumor immunology is that the tumor cells express inhibitory molecules and anti-inflammatory cytokines to escape from anti-tumor responses.[1] However, it becomes more intriguing since the costimulatory molecules can also be found on the tumor cells. Acute myeloid leukemia (AML) is a fine example for this paradoxical situation. The potent costimulatory molecules of B7 superfamily, B7-2 (CD86) and B7-H2 (ICOS ligand, ICOSL), have been detected on the subpopulations of myeloid leukemia cells.[3-5] These molecules are critical for T cell activation and, therefore, for successful anti-tumor immune responses. CD28 is the receptor for B7-1 (CD80) and B7-2 (CD86) which are critical costimulatory molecules for naïve helper T cells' priming.[6] On the other hand; B7-H2 serves as a ligand for CD28 and the inducible costimulator (ICOS) that provides signals for the persistence of T cell activation.[6] Intriguingly, in several independent studies, the presence of B7-2$^+$ and/or B7-H2$^+$ AML subpopulations had been determined as of strong prognostic value that can indicate poor clinical outcomes such as hyperleukocytosis, short disease-free or relapse-free survival.[3-5]

In our study, we conditioned a well-characterized myeloid leukemia cell line, HL-60, and were able to model the interaction between B7-2- and/or B7-H2-expressing leukemia cells and helper T cells.[7] Our data was also supported by other myeloid leukemia cell lines. Under suboptimal stimulation of T cell receptor (TCR) complex, myeloid leukemia cells generated potent costimulatory signals that are required for helper T cell responses.[7] Upregulation of T cell activation markers (CD154, CD25, and CD69), expansion, and secretion of Th1 and Th17 cytokines (IFN-γ, TNF-α, and IL-17A) was the result of helper T and AML cells' encounter, where costimulation was largely owed to B7-2$^+$ sub-population (Fig. 1).[7] A similar phenomenon was also observed with basal-like breast cancer (BLBC) cells (*manuscript in press*).

Provoked by the leukemia or BLBC cells, T cell responses resulted in a quick change in the expression of B7 ligands on cancer cells. The ligands for programmed cell death-1 (PD-1) receptor, B7-H1 (PD-L1) and B7-DC (PD-L2) were induced on the leukemia cells.[7] The inhibitory receptor PD-1 is expressed by activated T cells and mediates the regression of immune responses mainly via interfering with CD28-derived costimulatory signals.[8] On the other hand, when costimulatory signals are simultaneously delivered, the inhibitory effect of PD-1 can be weakened.[8,9] Nonetheless, the expression of costimulatory B7-H2 molecule was also downregulated on leukemia cells.[7] Collectively, after the initial engagement with helper T cells, the leukemia cells gained an inhibitory phenotype. We also confirmed that these cells were able to diminish T cell responses and might direct helper T cell differentiation towards regulatory (Treg) phenotype (Fig. 1).

Tumor cells hide from immune recognition and/or cope with the immune attack.[1] In other words, tumor cells which can successfully evade the anti-tumor immunity may emerge as a consequence of adaptation to the selective pressure applied by the immune system.[1] Our results demonstrate the rapid adaptation capacity of the leukemia cells in response to anti-tumor immune responses.[7,10] In our experience, only a small sub-population of leukemia cells were carrying B7-2 and/or B7-H2 molecules whereas it should be noted that many leukemia cells, expressing B7-2 or not, adopted this *de novo* immunosuppressive character.[7] Thus, during immune evasion, tumor cells may benefit from being composed of heterogeneous sub-populations.

In conclusion, AML or BLBC cells can directly motivate helper T cell responses, whereas in return, these tumor cells quickly alter their immune phenotype and resist to immune attack. The results obtained from our *in vitro* model may also have implications for immunotherapeutic approaches in AML or BLBC.[7,10]

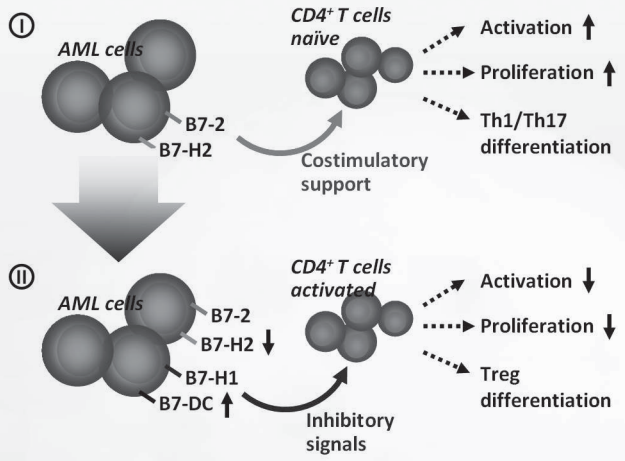

Figure 1. The costimulatory interaction between helper T cells and AML cells. I. The initial engagement with AML cells that harbor B7-2 (CD86) and/or B7-H2 (ICOSL) subpopulation provokes T cell responses and Th1/Th17 differentiation. II. In return, helper T cell responses modulate the expression of B7 family molecules on AML cells: decrease in B7-H2, and upregulation of B7-H1 (PD-L1) and B7-DC (PD-L2) expression. Subsequently, those AML cells gain immunosuppressive capacity, hamper helper T cell responses and favor regulatory T cell differentiation especially through PD-1 pathway.

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THE IMMUNE CONTEXTURE OF PRIMARY AND METASTATIC HUMAN TUMORS

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In the vast majority of cancer types, as demonstrated and exemplified in colorectal cancer (CRC), a high density of $CD8^+$ T cells in the tumor microenvironment correlates with a good prognosis for the patient. However, an opposite correlation has been reported in primary renal cell carcinoma. Our team studied the immune infiltrates of pulmonary metastases from CRC and renal clear cell carcinoma (RCC). We report that despite the advanced stage of the disease in these patients, the immune infiltrate of pulmonary metastases remains a prognostic factor. As in the primary tumors, a high density of $CD8^+$ T cells correlates with good prognosis for CRC metastases, while it correlates with a bad prognosis for RCC metastases. Moreover gene expression analyses reveal a higher expression of genes linked to Th2 orientation, inflammation, angiogenesis and immunosuppression in RCC than in CRC metastases (Remark et al., Clin Cancer Res., 2013).

Similar opposite effects were obtained applying immune gene signatures on the RCC dataset from The Cancer Genome Atlas and from the CRC dataset from the Moffit Cancer Center (Becht E *et al.,* unpublished). Thus a clear shift of the prognostic value associated to the expression of lymphocyte specific genes from favourable in CRC datasets to detrimental in RCC datasets was found compared to the distribution of all the other genes ($P=10^{-14}$ for CRC and $P=10^{-5}$ for RCC, Fisher's exact test) (Giraldo et al., Current Opinion Immunol., 2014). Therefore in most cancer types, a strong infiltration of memory Th1/cytotoxic T cells, likely educated in adjacent tertiary lymphoid structures (Goc et al., Cancer Research, 2014), correlates inversely with VEGFA, IL-6, STAT-3 expression and is associated with favourable prognosis. In RCC a strong infiltration of $CD8^+$ T cells is associated with a strong expression of VEGFA, STAT-3, IL-6, TNF-α, TGF-β and IL-10 and poor survival. These results highlight a novel tumour cell-dependent immune contexture that predicts patient's clinical outcome. Identifying which factors and pathways are critical for the RCC-related detrimental impact of $CD8^+$ T cells on prognosis will be a major breakthrough enabling the identification of new immunomodulatory drug targets and guide for immunotherapy protocols.

MONOCYTE DERIVED SUPPRESSOR CELLS AS TARGETS FOR TUMOR IMMUNOTHERAPY

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The ability of tumor-specific cytotoxic T cells and natural killer cells to eliminate cancers is hindered by large numbers of immunosuppressive cells present in the tumor microenvironment. Monocytic myeloid-derived suppressor cells constitute the majority of these tumor infiltrating leukocytes and are key contributors to the immunosuppressive milieu. MDSC arise in the bone marrow from myeloid progenitors and are present at high frequency in patients with cancer.

Murine mMDSC express TLR9 and respond to stimulation with CpG oligonucleotides (TLR9 agonists) by differentiating into tumoricidal macrophages. In vivo administration of CpG ODN slows/prevents the growth of tumors in mice, an outcome linked to the increased activity of tumoricidal T cells. Human mMDSC express TLRs 2, 7 and 8 (but not 9) and are induced to differentiate into macrophage when stimulated via the relevant Toll-like receptors. Agonists targeting TLR 1/2 (such as PAM3) induce mMDSC to mature into immumosuppressive M2-like macrophage whereas agonists targeting TLR 7/8 (such as R848) cause the same precursors to mature into tumoricidal M1-like macrophages.

We analyzed the changes in gene expression of mMDSC cultured with PAM3 vs R848. 94% of the genes up-regulated by PAM3 were also activated by R848. These common genes were associated with the maturation of mMDSC into M2-like macrophage. Ingenuity Pathway Analysis showed that TNF and to a lessor extent IL6 dominated the regulatory pathways that controlled the expression of these genes - a finding consistent with data from murine studies showing that TNF plays a central role in causing MDSC to mature into macrophage.

By comparison, R848 up-regulated many more genes than PAM3. Those that were uniquely activated by R848 presumably drove M1 macrophage maturation. IPA analysis of that gene subset showed that IL6 and TNF continued to play a role but identified IL12 as a unique and key regulator of nearly half of the genes preferentially stimulated by R848. We conclude that the phenotype of macrophage generated by TLR mediated maturation of mMDSC is fluid, with either M1- or M2-like macrophage being generated by interactions between IL6, TNF and IL12.

MODULATION OF ALLERGEN-SPECIFIC IMMUNE RESPONSES: NOVEL CONCEPTS, TOOLS AND PLATFORMS

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IgE-associated immune reactions affect more than 25% of individuals within our population. Consequently, the high prevalence of allergic diseases poses an enormous economic burden on the health care systems especially also due to the chronicity of the disease. Thus, there is a strong need for novel approaches towards allergy research and treatment. T-lymphocytes play a central role in the pathogenesis of allergic diseases. During the sensitization phase, priming of allergen-specific $CD4^+$ T-helper 2 (T_H2) cells results in the production of the T_H2 cytokines interleukin(IL)-4 and IL-13, which - along with CD40L-CD40 mediated co-stimulation of B cells - are responsible for class switch recombination to IgE, allowing IgE production by B-cells/plasma cells. Other T_H2 cytokines favor the expansion and recruitment of innate effector cells such as mast cells and eosinophils to sites of allergic inflammation. Once primed, allergen-specific T-cells may also critically contribute to late phase reactions and allergic inflammation in target organs, e.g. in the airways and the skin. Because of the importance of T-cells and due to the paucity of appropriate human model systems we have resorted to cloning and functionally characterizing allergen-specific T-cell receptors (TCR). We have characterized several TCRs specific for the major pollen allergens of birch and mugwort, i.e. Bet v 1 and Art v 1, which represent clinically important allergens in the Northern Hemisphere. We could show that allergen-specific TCRs serve as basis for the large-scale generation of allergen-specific T-cells in a short period of time and independent of the allergic status of the donor and the allergy season. Significantly, allergen-specific TCR tg T-cells can be instructed to specifically impact on unfavorable (T_H2) effector cell responses. Recently, we have created a double transgenic animal model consisting of a human Art v 1-specific TCR (TRAV17/TRBV18) and human MHC molecules (HLA-DRA*01:01/-DRB1*01:01). Upon exposure, these humanized 'allergy mice', but not control mice, specifically respond with airway hyperreactivity (AHR) and production of allergen-specific immunoglobulins. Of significance, increased Treg numbers prevent the occurrence of AHR in 'allergy mice'. Humanized 'allergy mice' represent valuable tools to investigate the pathophysiology, prevention and cure of allergic diseases in a human-relevant context.

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INTERNATIONAL GUIDELINES FOR POSTTRANSPLANT ANTIBODY MONITORING IN ORGAN TRANSPLANTATION

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In organ transplantation, the introduction of the solid phase immunoassay technology radically changed the practice of HLA antibody monitoring. Precise identification of antibody specificities in complex sera of sensitized patients and monitoring of low levels of donor-specific HLA antibodies in the posttransplant phase became possible. However, at the same time new technical problems and great variation emerged in the interpretation of test results, indicating a need for standardization. In May 2012, The Transplantation Society (TTS) recruited a panel of laboratory and clinical experts to discuss emerging issues that are associated with antibody testing in organ transplantation. Subsequently, the same group of experts formulated "Consensus Guidelines" which were published in the January 15, 2013 issue of Transplantation. I will provide a summary of the TTS recommendations formulated in this international effort on the standardization of "posttransplant" antibody monitoring in kidney transplantation.

CHARACTERISTICS OF PRIMARY IMMUNODEFICIENCIES IN TURKEY

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Primary immunodeficiency diseases (PID) are a heterogeneous group of inherited disorders of the immune system, predisposing individuals to recurrent infections, allergy, autoimmunity, and malignancies. The phenotypic diversity of PIDs is contributed by the heterogeneity of mutations that may affect single genes and that may variably affect protein expression and function. Clinical descriptions have already been made for more than 200 PIDs [1-3], for which over 150 forms of PID have been molecularly characterized and this number is still expanding.

The International Union of Immunological Societies (IUIS) classifies diseases into 8 major groups and subgroups [4]: combined T- and B-cell immunodeficiencies, predominantly antibody deficiencies, other well-defined immunodeficiency syndromes, diseases of immune dysregulation, congenital defects of phagocyte number, function, or both, defects in innate immunity, autoinflammatory disorders, and complement deficiencies.

Epidemiological studies have shown wide goegraphical and racial variations in the prevalence and the pattern of immunodeficiency diseases. Several PID patient registries have been released in different countries [5-15]. These registries helped in determining the frequency of PID in these countries. The ESID patient registry is the largest of all and a secure, internet based patient registry, which combines both clinical and laboratory data of PID patients. The ESID database consists of more than 200 disease specific registries, which are grouped into eight main categories.

Each different PID has its own sub-registry. There is no national registry of PID's in Turkey.

A population prevalence of diagnosed PID in the United States at approximately 1 in 1,200 persons [14]. Data from the Kuwait National Primary Immunodeficiency Registry provides a PID prevalence estimate of 11.98 per 100 000 children and an estimated occurrence of PID in one in 1000 live births [8]. The Australian Society for Clinical Immunology and Allergy's PID registry provided a prevalence of 4.9 per 100 000 in Australia and New Zealand combined [9]. The prevalance of PIDs in the general population has not been identified clearly in Turkey.

This study was performed to determine the frequency, characteristics and clinical course of various PID disorders among patients diagnosed over six years at the Department of Pediatric Immunology, Uludag University Medical Faculty and Department of Pediatric Immunology, Ege University Medical Faculty. These two centers are the major contributors reporting PID patients to ESID database from Turkey.

The European Society for Immunodeficiencies (ESID) has developed an internet-based database for clinical and research data on patients with PID. This study aimed to provide a minimum estimate of the prevalence of each disorder and to determine the clinical characteristics and outcomes of patients with PID in Turkey.

Clinical features of 1435 patients with primary immunodeficiency disorders are registered in ESID Online Patient Registry by the Pediatric Immunology Departments of the Medical Faculties of Uludag University and Ege University Between 2004 and 2010. These two centers are the major contributors reporting PID patients to ESID database from Turkey.

Results: Predominantly antibody immunodeficiency (69%) were the most common category followed by autoinflammatory disorders (13%), other well defined immunodeficiencies (9%), congenital defects of phagocyte number, function or both (4 %), combined T and B cell immunodeficiencies (2%), defects in innate immunity (2%), and diseases of immune dysregulation (1%). Patients between 0-18 years of age constitued 94 % of total and the mean age was 9.2±6 years. The consanguinity rate within the registered patients was 14.3 % (188: 1130). The prevalance of all PID cases ascertained from the registry was 30.5/100.000. Forty-two of seventy five patients died from infections with highest mortality for severe combined immunodeficiencies and ataxia-telangiectasia.

To promote awareness of PID among the medical profession and sectors of the general public are required if premature death and serious morbidity due to late diagnosis of the wider spectrum of PID are to be avoided.

SEVERE COMBINED IMMUNODEFICIENCIES: CLINICAL EXPERIENCE

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Severe combined immunodeficiency (SCID) is an heterogeneous group of primary immunodeficiency resulting from inherited defects of the cellular and humoral immune system. A variety of mutations that affect the genes involved in development and maturation or activation of T cells may cause may an SCID phenotype. The defects can be grouped into cytokine signaling defects (gc, JAK3, and IL7Ra deficiency), T-cell receptor and signaling defects (CD3 subunits, ZAP70, CD45, coronin1A deficiency), VDJ recombination defects (RAG1/2, radiosensitive SCID), and metabolic defects (adenosine deaminase (ADA) deficiency, reticular dysgenesis).

Infants born with SCID typically appear normal at birth, but are at high risk of serious infections after waning of maternal antibody. If untreated, SCID has 100% mortality. Treatment is generally hematopoietic stem cell transplantation (HSCT), although gene therapy has been successfully used in some forms of SCID, such as adenosine deaminase or IL-2 receptor gamma defect. HSCT is a potentially curative measure that should be considered as early as possible due to high rate of complications of severe infections.

Because early diagnosis of SCID is critical, detection of SCID through newborn screening with the T-cell receptor excision circle (TREC) assay is the ideal approach.

In this speech we will present the characteristics of patients with SCID and our experience on hematopoietic stem cell transplantation performed to the patients with SCID.

IMMUNOREGULATION AND IMMUNITY TO HELMINTH PARASITES

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Helminth parasites are highly prevalent in humans and animals throughout the world today. Their success reflects a mastery of immune evasion, down-regulating inflammatory responses even to the extent of blocking autoimmunity and allergies to unrelated antigens. To analyse the mechanistic basis of these immunological effects, we are studying a model intestinal nematode *Heligmosomoides polygyrus*, which is related to human hookworm parasites. Infected mice show a profound change in immune status, with suppression of allergic airway reactivity, as well as diminished autoimmune and other inflammatory responses. Regulatory cell populations expand in infected mice, including both natural and adaptive Foxp3$^+$ Tregs and regulatory B cells, which inhibit allergic inflammation and autoimmune disease when transferred to uninfected animals. The immunoregulatory effects can be recapitulated with soluble products (termed HES) secreted by live parasites in vitro. HES induces de novo Foxp3 expression and Treg function in naive peripheral murine T cells, acting through a parasite-encoded mimic of host TGF-β. HES can also block TLR-stimulated release of IL-12 by dendritic cells, and the release of IL-33 by epithelial cells, in a TGF-β-independent manner. The immunoregulatory properties of *H. polygyrus* can be neutralized by immunization of mice with HES in adjuvant; vaccination elicits 100% sterile immunity, accelerating immune cell extravasation and trapping tissue-phase larvae in the wall of the small intestine. Immunity is dependent on the induction of antibodies, and passive transfer of serum IgG from vaccinated mice confers significant protection, but not sterilising immunity, on recipient mice. Immunity also requires an activated myeloid cell population for which IL-4R-mediated signalling is essential. Two further mediators which are required for the cellular component of immunity are IL-25 and macrophage migration inhibitory factor (MIF), emphasising the contribution of innate immunity. Hence, secreted worm immunomodulators not only offer novel therapies for immunopathological disorders, but also effective targets for anti-parasite vaccines to generate protective immunity to helminth infection.

HOST-PATHOGEN INTERACTIONS IN THE CONTEXT OF MALARIA

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Malaria is an important infectious disease, still risking around 3.5 billion people's life every day. Nearly half million people died due to malaria and related complications in 2012 (http://www.who.int/malaria/media/world_malaria_report_2013/en/).

In my lab, we are interested in host-pathogen interactions, trying to have answer the questions how host recognize, response and/or coop with *Plasmodium* parasites. We investigate the role of both innate and adaptive immunity in response to Plasmodium parasites and protective mechanism (s) elucidated by host.

We've recently developed new experimental approaches for the investigation of malaria immunopathology by utilizing cutting-edge technological advances in imaging field. We've combined ultra-high field MRI and multi-photon live imaging to visualize parasites as well as host responses to reveal immunopathology of experimental cerebral malaria. For example, we've recently found previously unnoticed, truly overlooked location affected first by parasites and related events. We'll discuss our recent results in this talk.

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THE INTERPLAY BETWEEN INFLAMMATION AND NEURAL STEM CELLS: ZEBRAFISH TAKES THE HEAT UP A NOTCH FOR REGENERATIVE THERAPIES

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Etymologically, inflammation denotes a metaphoric blaze as caricaturized for sumptuous mythological creatures. A tale on Phoenix for instance narrates the fire devouring its body into ashes, from which a new bird arise anon. Thus, the state of torrid heat in the myths is both devastating and revitalizing. In biological systems, the situation is in fact not utterly different. Inflammation entails a complex set of defense mechanisms acting in concert to restore the homeostatic balance in organisms upon damage or pathogen invasion. This immune response comprises of the activity of various immune cells, and is a double-edged sword as evidence exists for detrimental and beneficial consequences. Additionally, majority of the chronic diseases involve an unremitting phase of inflammation due to improper resolution of the initial pro-inflammatory response that impinges on the stem cell behavior. Therefore, understanding the effect of inflammation on stem cell activity is not only important to further delineate the etiology of diseases, but also essential for designing regenerative therapies by micromanipulating the inflammatory milieu to offset the negative effects and maximize the beneficial outcomes. In my talk, I will give an overview of the crosstalk between the inflammatory scene and tissue-resident neural stem cells. I will focus on our efforts to unravel the molecular mechanisms of neural stem cell activation through inflammation, and the applicability of this understanding on translational medicine.

BIOCHEMICAL ANALYSIS OF LYMPHOCYTE LINEAGE COMMITMENT USING THE LOCUS-SPECIFIC CHROMATIN IMMUNOPRECIPITATION TECHNOLOGIES (iChIP AND enChIP)

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Comprehensive understanding of mechanisms of transcriptional regulation requires identification of molecules bound to promoter and regulatory regions of genes of interest *in vivo*. To perform biochemical and molecular biological analysis of specific genomic regions, we developed the insertional chromatin immunoprecipitation (iChIP) technology to purify the genomic regions of interest (1-6). Specific genomic regions tagged with the recognition sequences of an exogenous DNA-binding protein such as LexA are subjected to affinity purification in iChIP. iChIP is a comprehensive approach to purify specific genomic regions of interest to identify interacting molecules including genomic DNA, proteins, RNA, and others, with an emphasis on non-biased search using next-generation sequencing (NGS), microarrays, mass spectrometry (MS), and other methods. In addition, this approach is not restricted to cultured cell lines but easily extended to organisms *in vivo*.

We applied iChIP combined with SILAC (iChIP-SILAC) to direct identification of proteins interacting with the promoter region of the single-copy chicken *Pax5* gene, which is the master transcription factor in B cell development. By comparing B cells with a macrophage-like cells trans-differentiated by ectopic expression of C/EBPβ, iChIP-SILAC identified proteins interacting with the *Pax5* promoter in a B cell-specific manner. Loss-of-function of the identified proteins induced decrease in Pax5 expression. Thus, our analysis revealed that the identified proteins are functionally required for B cell-specific expression of Pax5. Furthermore, these results showed that iChIP-SILAC would be a useful tool for identification of proteins interacting with even a single-copy locus in cells of multicellular higher eukaryotes *in vivo*. In addition, we used iChIP-Seq to identify genome-wide intra- and intergenomic interactions with the *Pax5* promoter. The significance of these interactions in Pax5 expression will be discussed.

In addition, we recently developed engineered DNA-binding molecule-mediated chromatin immunoprecipitation (enChIP) to purify the genomic regions of interest (7, 8). In enChIP, specific genomic regions are tagged with engineered DNA-binding molecules such as TAL proteins and the CRISPR system consisting of a catalytically inactive form of Cas9 (dCas9) plus guide RNA (gRNA) for biochemical purification. I will discuss applications of the enChIP technology to elucidation of molecular mechanisms of genome functions in the immune system.

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GENETICS AND IMMUNOPATHOGENESIS OF TAKAYASU'S ARTERITIS

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Takayasu's arteritis (TAK) is a chronic, inflammatory, large-vessel vasculitis affecting the aorta and its major branches. It is mainly observed in young (<40 years) and female (>80%) patients. Although more prevalent in Asia, the distribution of the disease is worldwide with a low prevalence (0.5-3/million) [1, 2].

The immune response in TAK is granulomatous in nature with a Th1 (and possibly Th17) type response. Among various cytokines studied, IL-6, IL-8 and IL-18 levels are increased in sera, especially in active disease. The etiopathogenesis of TAK is unknown, but M. tuberculosis (TB) infection is implicated for many years with co-existent case reports and immune responses to TB antigens, PPD and mycobacterial heat-shock proteins. TB-associated IS6110 and HupB gene sequences in aorta specimens is recently shown to be increased (70%) in TAK patients compared to atherosclerosis. However, we have shown that although PPD response is higher in TAK patients (63% vs 41% positivity in controls), a TB-specific interferon-gamma assay (Quantiferon), showed no increased active or latent TB infection (22% positivity both in TAK and controls)[3].

Genetic studies in TAK started with HLA-typing and single nucleotide polymorphism (SNP) analysis of various candidate genes. Among the proinflammatory and Th1 type cytokines, several SNPs in IL-2, IL-6 and IL-12B genes are shown to be increased in the Turkish population [4]. However, HLA-B*52 is currently the most prominent genetic marker associated with TAK (OR=2.2-7.5) in different ethnicities. B*52 has also shown a significant association with TAK (OR=3,7) in Turkey [5]. In contrast, the distribution of B*51 (an HLA-B*5 allele with only 2 aa difference and strongly associated with Behcet's disease) did not differ between the TAK patients and controls, suggesting a possible specific role of the "antigenic peptide" presented by B*52 allele. The presence of B*52 is decreased in late-onset (>40 years) patients (12%, OR=0,43) and type I angiographic disease with limited aortic involvement (13.1%, OR=0,43).

Recently, first whole-genome genetic studies (GWAS) are performed in TAK, one a collaborative study of Turkish and USA patients and another one from Japan with "Immunochip" arrays, screening approximately 200.000 SNPs, shown to be previously associated with immune-mediated diseases [6, 7]. Both studies demonstrated IL-12B, in addition to HLA-B*52 as an additional risk factor for TAK. IL-12B SNPs are previously associated with type-1 diabetes, psoriasis, asthma and SLE. Collaborative study also demonstrated other susceptibility loci in HLA-DQB1/HLA-DRB1 and FCGR2A/FCGR3A regions. Fc gamma receptors (FCGR) bind to Igs attached to infected cells or pathogens. FCGR-associated SNPs are shown to be associated with infections such as HIV, cryptococcia, meningitis and immune-mediated diseases such as giant-cell arteritis, ulcerative colitis, rheumatoid arthritis and SLE.

The prognosis of TAK is quite variable (surgery: 12-50%, mortality: 3-17%) in different published case series. Corticosteroids and immunosuppressive treatments (mainly methotrexate and azatioprine) are accepted to be effective, but have serious long-term side effects. In refractory cases, monoclonal anti-cytokine antibodies against TNF**a** and IL-6 seem to be effective, suggesting their major role in pathogenesis. Further collaborative studies are required to clarify the role of genetic, enviromental and immune/inflammatory mechanisms in the etiopathogenesis of TAK.

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PATHOGENESIS AND ROLE OF IL-1 SIGNALING IN KAWASAKI DISEASE CORONARY ARTERITIS, VASCULITIS AND ANEURYSMS. LESSONS LEARNED FROM THE KAWASAKI DISEASE MOUSE MODEL OF VASCULITIS

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Background **-** Kawasaki disease (KD) is the most common cause of acute systemic vasculitis and acquired cardiac disease among US children particularly affecting the coronary arteries, causing coronary artery aneurysms (CAA) in 15-25% of untreated patients. KD is also potentially an important cause of long-term cardiac disease in adult life. In a *Lactobacillus casei* cell wall extract (LCWE)-induced mouse model of KD we have already shown that coronary arteritis and vasculitis was an IL-1-driven process. Aneurysms due to KD have been reported in other systemic arteries including the axillary, subclavian, abdominal, renal and iliac arteries; however, their prevalence is low.

Methods and Results. We investigated the incidence and progression of abdominal aorta involvement in the KD mouse model by measuring the diameter of abdominal aorta at 2 and 5 weeks following LCWE injection. Over 80% of the mice developed significant dilation of abdominal aorta at 2 wks with progressively higher dilatation at 5 wks following LCWE injection. Some mice showed fusiform and saccular abdominal aneurysms, as well as dilations of iliac and renal aorta. Histopathology by H&E and Elastin staining showed significant intimal proliferation, massive myofibroblastic proliferation that penetrates and breaks the elastin layer, significant inflammatory cell infiltration into media and adventitia. Immunofluorescence staining also revealed the infiltration of large number of neutrophils and dendritic cells. We also detected the small population of CD4+, CD8+ T cells and macrophages in the lesion. In addition, IL-1R KO or IL-1beta KO-mice were completely protected not only from the coronary arteritis but also from the abdominal aorta dilatation and aneurysm formation.

Conclusions **-** We describe for the first time the presence of abdominal aorta dilatation and aneurysms in the LCWE-induced KD mouse model. The incidence of this finding was over 80% and the dilatation progressed with time, suggesting that in children with KD the incidence of abdominal aorta dilatation maybe higher than what has been appreciated until now. Our findings demonstrate that LCWE-induced KD model abdominal aorta dilatation, aneurysm and inflammation is also IL-1 signaling-dependent and blockade of IL-1 signaling molecules maybe a promising therapeutic target for KD coronary arteritis and systemic arterial injury as well as aneurysm formation.

ORAL PRESENTATIONS

ABSTRACT REF.: 042

ENHANCED SENSITIVITY OF COLON TUMOR CELLS TO NATURAL KILLER CELL CYTOTOXICITY AFTER MILD THERMAL STRESS IS REGULATED THROUGH HSF1 MEDIATED EXPRESSION OF MICA

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PURPOSE: Previously we showed that mild thermal stress increased natural killer (NK) cell mediated tumor cytotoxicity and that this could be blocked by anti-NKG2D or anti-MICA (major histolocompatability complex (MHC) class I related chain A) antibodies. Here, we investigated the role of the transcription factor heat shock factor 1 (HSF1) in thermal regulation of MICA expression in tumor cells in vitro and in vivo.

METHODS: Hyperthermia experiments were conducted in vitro and in mice using a target temperature of 39.5 C. Apoptotic cells and NK cells in situ were visualised by use of the TUNEL assay or expression of NKp46 respectively. Using Colo205 cells, HSF1 message was blocked utilising siRNA while luciferase reporter assays were used to measure the activity of the MICA promoter in vitro. Cell surface MICA was measured by flow cytometry.

RESULTS: Following whole body hyperthermia (WBH), tumor tissues showed an increase in NK cells and apoptosis. Mild thermal stress resulted in a transient increase in surface MICA and enhanced NK cytotoxicity of the Colo205 colon cancer cell line. Silencing (mRNA) HSF1 expression in Colo205 cells prevented the thermal enhancement of MICA message and surface protein levels, with partial loss of thermally enhanced NK cytotoxicity. Mutations of the HSF1 binding site on the MICA promoter implicated HSF1 in the thermal enhancement of MICA. Some, but not all, patient-derived colon tumour derived xenografts also exhibited an enhanced MICA message expression after WBH.

CONCLUSIONS: Up-regulation of MICA expression in Colo205 cells and enhanced sensitivity to NK cell killing following mild thermal stress is dependent upon HSF1.

Keywords: Natural Killer Cells, colon tumors, fever range hyperthermia, HSF-1, MICA, NKG2D

ABSTRACT REF.: 086

ENHANCED IMMUNOSTIMULATORY PROPERTIES OF EXTRACELLULAR VESICLES HORBORING DIFFERENT TLR LIGANDS

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Extracellular vesicles are naturally occurring membranous particles secreted from several cells. Based on their cargo, upon internalization, they can regulate cell physiology as well as mediate immune responses. In this study, we aimed to i) characterize, ii) explore the immunostimulatory activities of EVs isolated from different cell lines and iii) their potential upon incorporating with different TLR ligands as drug delivery system for treatment of diseases. EVs were isolated from RAW264.7 and E.G7 cell supernatants by differential centrifugation, filtration and ultracentrifugation and were loaded with D-type ODN. Physicochemical properties of EVs were analyzed either by atomic force microscopy (AFM) or by dynamic light scattering (DLS). Labeled EVs were incubated with mouse splenocytes to evaluate their uptake and internalization by splenic macrophage, B and T and dendritic cells via FACS analysis using specific murine antibodies against these cell types. In vivo biodistribution of EVs were analyzed 24 hour post ip injection by FACS from spleen, PEC and lymph node cells. In vitro stimulatory activity of D-ODN loaded EVs or EVs incubated with TLR ligands were tested on mouse splenocytes by cytokine ELISA. AFM and DLS confirmed that variuos forms of EVs (i.e. exosomes or microparticles) could be isolated from RAW264.7 and E.G7 cell lines. Uptake and internalization studies on mouse splenocytes showed that the type of target cells that these EVs are accumulated is source cell specific. According to in vivo biodistribution studies, PEC specific macrophages are able to take-up more EVs than other lymphoid tissues independently from source of the EV. In vitro stimulation assays revealed that D-ODN encapsulation into EVs significantly increased IL6, IL12, IFNg and IFNa production in comparison to that of free ODN counterpart. Furthermore, TLR ligand associated EVs, compared to their free forms pronounced IL6 and IL12 production by splenocytes. In conclusion, EVs isolated from different cell sources induced different immunostimulatory activities on immune cells. Also, these EVs can be used as potential drug carrier vesicles harboring clinically important ligands. These forms of natural carriers can be harnessed for more effective immune therapy against cancer or treatment of infectious diseases.

Keywords: Extracellular Vesicle, TLR, CpG ODN, Innate Immune Response, Vaccine

ABSTRACT REF.: 111

HELICOBACTER PYLORI SEROPOSITIVITY MIGHT SUPPORT THE IMMUNE TOLERANCE IN COLORECTAL CANCER CASES

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Persistent Helicobacter pylori (H.pylori) seropositivity is associated with risk of colorectal cancer. Although host displays a vigorous innate and adaptive response against the bacterium, H.pylori escape from immune response. Aim of this study is to evaluate how H.pylori is able to evade the immune response and whether it enhances systemic immune tolerance against colorectal cancer. Ninety-seven adults with colorectal cancer and 108 cancer free patients with extra-digestive diseases were involved in this study. Each group was assigned into two subgroups according to H.pylori IgG seropositivity. Exposure to H. pylori was determined by serum IgG test (ELISA). Serum neopterin, tryptophan, kynurenine, nitrite and urinary biopterin concentrations were measured. Serum indoleamine 2,3-dioxygenase activity was estimated by kynurenine to tryptophan ratio. The frequencies of increased serum kynurenine-tryptophan ratio of H.pylori seronegative and seropositive colorectal cancer groups were estimated by compar-

ing with the average amount of serum IDO activity of H.pylori seronegative tumor-free patients. In order to estimate tetrahydrobiopterin, its oxidation product, urinary biopterin and creatinine levels were assayed. While serum tryptophan levels were decreasing, kynurenine-tryptophan ratio significantly increased in both H.pylori seronegative and seropositive colorectal cancer patients. Although no statistically significant difference was found between IDO activities of H. pylori seronegative and seropositive cancer patients, the frequency of increased IDO activity was higher in seropositive subgroup. The decrease in serum nitrite and albumin levels of H.pylori seropositive cancer cases might be attributed to the excessive reactive oxygen radical production. As a conclusion, H.pylori seropositive colorectal cancer patients with significantly higher kynurenine-tryptophan ratio suggested that H.pylori might support the immune tolerance leading to cancer development and/or spreading, even though in patients without an apparent upper gastrointestinal H.pylori disease.

Keywords: Helicobacter pylori, tryptophan, kynurenine, neopterin, colorectal cancer, immune tolerance

ABSTRACT REF.: 056

DIFFERENTIAL ACTIVATION OF IMMUNE CELLS BY COMMENSAL VERSUS PATHOGEN-DERIVED BACTERIAL RNA

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Immunological mechanisms contributing to distinguishing signals derived from commensal versus pathogenic bacteria is an active area of research and recent evidence suggests that commensal and pathogens may express different variants of pathogen associated molecular patterns (PAMP). We hypothesized that as a major member of PAMP, bacterial RNA (bacRNA) originating from commensals versus pathogens may possess distinct immunostimulatory activities, enabling their discrimination by the immune system. To test this hypothesis, human PBMCs, purified human neutrophils, and 2 distinct reporter cell lines stably expressing the endosomal ssRNA sensor TLR7 or the cytosolic sensors RIG-I and MDA-5 were stimulated with various doses of human commensal or pathogen-derived purified RNAs as such or following their complexation with the transfection reagent Lipofectamine 2000. Results showed that commensal but not pathogen derived bacRNA induced type-I IFN secretion from hPBMC and RIG-I/MDA-5 expressing B16 IFN-reporter cell line. Consistent with their type I interferon stimulating activities, commensal bacRNAs stimulated significant levels of IP-10 production from hPBMC, whereas pathogen bacRNAs were ineffective. In contrast, RNAs derived from pathogens triggered significantly higher levels of IL-6 and IL-1β production from hPBMCs even in the absence of transfection. Interestingly, pathogen bacRNAs were significantly more stimulatory when tested in a HEK TLR7 reporter cell line. Purified human neutrophils responded to pathogen derived RNAs but not to commensal derived RNAs by secreting IL-8 in the absence of neutrophil extracellular trap (NET) formation. These results suggest that commensals and pathogens may possess RNAs with sufficiently distinct structural features enabling their discrimination by immune cells. Since bacterial RNA was previously shown to be a signature of microbial vitality (a VitaPAMP), we next tested the vaccine adjuvant activities of commensal versus pathogen derived bacRNAs in mice immunized with the model antigen OVA. Results showed that pathogen but not commensal RNAs significantly boosted OVA-specific IgG2c titers in C57BL/6 mice to levels that was comparable to the polyI:C adjuvanted group.

In conclusion, our results show that pathogen and commensal bacterial RNA is recognised by distinct set of receptors and induce differential activation of immune cells. Structural differences associated with commensal versus pathogen derived RNAs, the route of entry of these RNAs and their resistance to plasma nucleases might be important factors that contribute to such distinct responses.

Keywords: Bacterial RNA, commensal, pathogen, Type-I IFN

ABSTRACT REF.: 018

MEMORY CD8+ T CELLS RESIDE AND REST IN CONTACT TO IL-7 PRODUCING STROMA CELLS IN MURINE BONE MARROW

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Memory (m) CD8+ T cells are important components of immune responses against recurrent infections and understanding their biology is crucial to improve their protective capabilities. It remains to be shown how and where mCD8+ T cells are maintained over long periods of time. mCD8+ T cells that were generated upon a specific immune response have been shown to populate in bone marrow (BM); however, their function and persistence have not been thoroughly investigated. In this study we aimed to elucidate whether mCD8+ T cells reside in distinct niches in BM as resting cells.

Our results show that following peptide immunization or LCMV infection the BM hosts equal, if not greater, numbers of Ag-specific mCD8+ T cells compared to the spleen indicating that BM is a major location for memory CD8+ T cell maintenance. A substantial number of both Ag-specific and non-specific memory phenotype CD8+ T cells in the BM express CD69, also known as "early activation marker". Despite this, the vast majority of BM cells are resting in terms of proliferation and gene expression suggesting that CD69 marks resident rather than activated cells. Memory phenotype CD8+ T cells reside next to BM stromal cells that produce IL-7, which is the major survival factor for mCD8+ T cells. In conclusion, our findings suggest that mCD8+ T cells reside in BM as resting rather than activated cells and are maintained in distinct survival niches involving IL-7 producing stromal cells.

Keywords: memory, CD8, bone marrow, CD69, LCMV, IL-7

ABSTRACT REF.: 058

MAGNIFIED AND PERSISTENT TH1-BIASED IMMUNITY MEDIATED BY EXOSOME VACCINE

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Exosomes are secreted biological nanovesicles and assumed plethora of physiological functions ranging from transport of cargo, regulating distant cell communication, and altering immune response. Accumulating evidence suggests exosomes are suitable candidate as delivery vectors. Efforts were directed either to engineer cells to express desired cargo in/on these secreted exosomes or induce physical complexing with candidate drugs or even use membrane-breaching techniques such as electroporation to generate exosomes harboring desirable cargo. Although, these techniques highlight the importance of utilizing exosomes as natural nanocarriers, they either depend on cell viability, leading to batch-to-batch variations thus lacking reproducibility or do not allow simultaneous high yield incorporation of more than one reagent within exosomes. Techniques that overcome these hurdles surely expand the biotherapeutic breadth of exosomes. Here we exploited therapeutic potential of externally loading purified exosomes with CpG motif expressing oligonucleotide together with a protein antigen as a cancer vaccine. The method aims to simultaneously load bio-molecular cargo within exosomes at high yield. This simple and mild approach allows simultaneous freeze-drying of pre-mixed vesicles and cargo, and upon subsequent rehydration, following lyophilization leads to ODN and protein internalization within exosomes with encapsulation efficiency over 70%.

CpG ODN loaded within exosomes yielded increased in-vitro activity as evidenced by IL6, or IL12 secretion from mouse spleen cells (up to 6 fold). Exosomes protected CpG ODN from digestion by DNase I up to 80%.It also facilitated faster and enhanced cellular entry mainly via scavenger receptor mediated internalization pathway thereby led to an increased IFNα production by intracellular cytokine staining. In order to demonstrate improved in-vivo activity, exosomes co-encapsulating D35 CpG ODN and antigen ovalbumin were tested as a potential vaccine vector. Animals that received Exo(D35+OVA) vaccine led to a magnified and persistent Th1-biased anti-OVA IgG responses (5 fold higher IgG2c/IgG1 ratio compared to CpG + OVA mixed group) that was sufficient to fully protect mice from EG-7 derived tumor challenge even after six months post-booster injection as opposed to free vaccine.

The present platform opens a new avenue to personalized cell-free therapeutic intervention and could be developed to harbor other therapeutically important molecules ranging from plasmid to mRNA or si/miRNA for more effective drug development in the clinic.

Keywords: Exosome, Vaccination, CpG ODN, Th1 immunization, Delivery Vesicle

ABSTRACT REF.: 009

LATENCY-ASSOCIATED PROTEIN ACR1 IMPAIRS DENDRITIC CELL MATURATION AND FUNCTIONALITY: A POSSIBLE MECHANISM OF IMMUNE EVASION BY MYCOBACTERIUM TUBERCULOSIS

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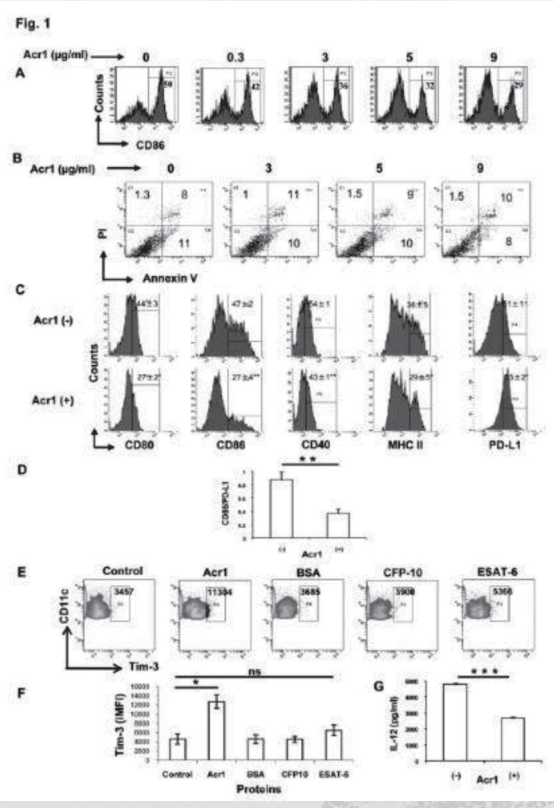
Mycobacterium tuberculosis (Mtb) in latently infected individuals survives and thwarts the attempts of eradication by the immune system. During latency, Acr1 is predominantly expressed by the bacterium. However, whether Mtb exploits its Acr1 in impairing the host immunity remains widely unexplored. Hence, currently we have investigated the role of Acr1 in influencing the differentiation and function of dendritic cells (DCs), which play a cardinal role in innate and adaptive immunity. Therefore, for the first time, we have revealed a novel mechanism of mycobacterial Acr1 in inhibiting the maturation and differentiation of DCs by inducing tolerogenic phenotype by modulating the expression of PD-L1; Tim-3; indoleamine 2, 3-dioxygenase (IDO); and interleukin 10. Furthermore, Acr1 interferes in the differentiation of DCs by targeting STAT-6 and STAT-3 pathways. Continuous activation of STAT-3 inhibited the translocation of NF-κB in Acr1-treated DCs. Furthermore, Acr1 also augmented the induction of regulatory T cells. These DCs displayed decline in their antigen uptake capacity and reduced ability to help T cells. Interestingly, Mtb exhibited better survival in Acr1-treated DCs. Thus, this study provides a crucial insight into a strategy adopted by Mtb to survive in the host by impairing the function of DCs.

Keywords: Tuberculosis, Acr1, dendritic cells, immunosuppression



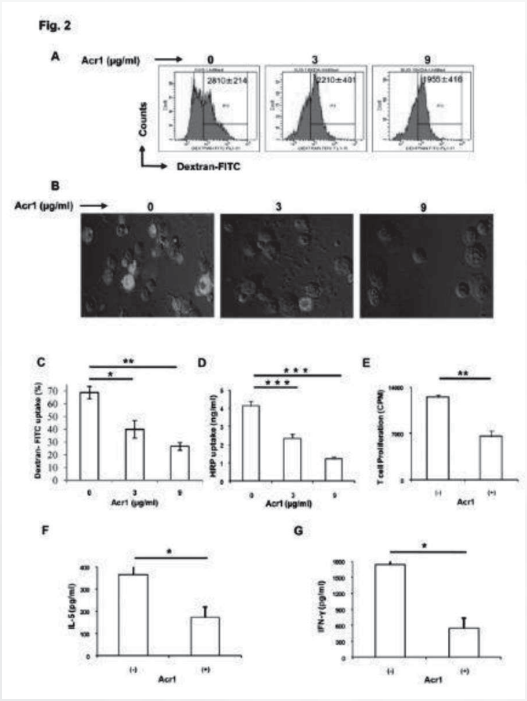


Fig. 2
A
Acr1 (µg/ml)
0
3
9
Counts
Dextran-FITC
B
Acr1 (µg/ml)
0
3
9
C
D
E
F
G
Acr1
(-)
(+)

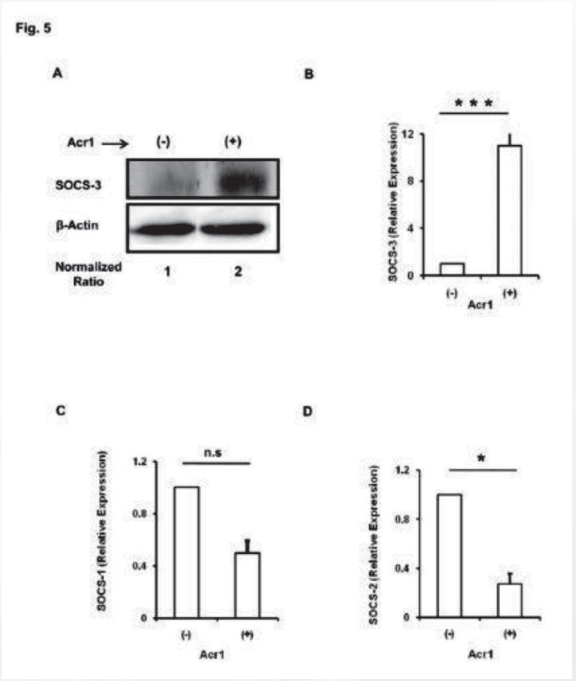


Fig. 5
A
Acr1
(-)
(+)
SOCS-3
β-Actin
Normalized Ratio
1
2
B
SOCS-3 (Relative Expression)
Acr1
C
SOCS-1 (Relative Expression)
n.s
D
SOCS-2 (Relative Expression)

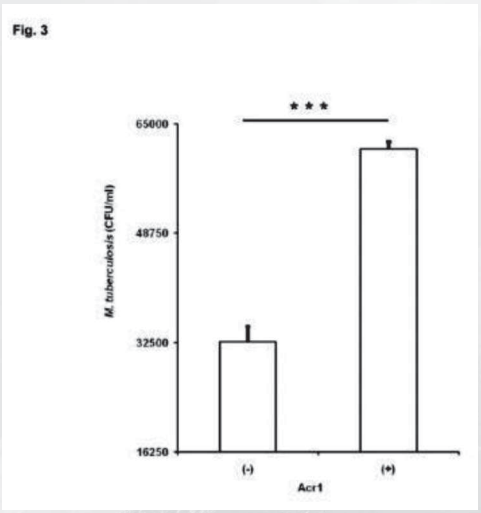


Fig. 3
M. tuberculosis (CFU/ml)
65000
48750
32500
16250
Acr1
(-)
(+)

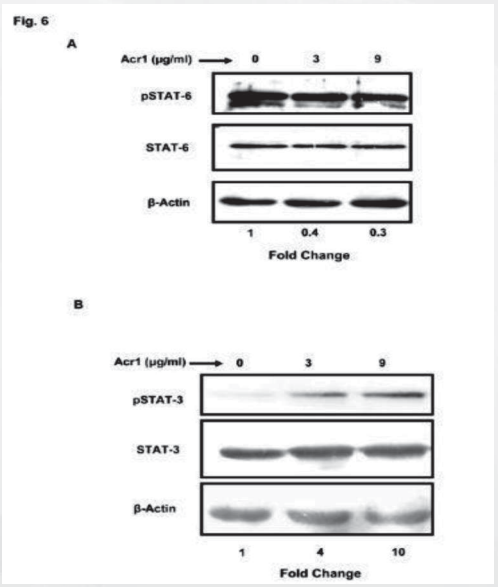


Fig. 6
A
Acr1 (µg/ml)
0
3
9
pSTAT-6
STAT-6
β-Actin
1
0.4
0.3
Fold Change
B
pSTAT-3
STAT-3
1
4
10

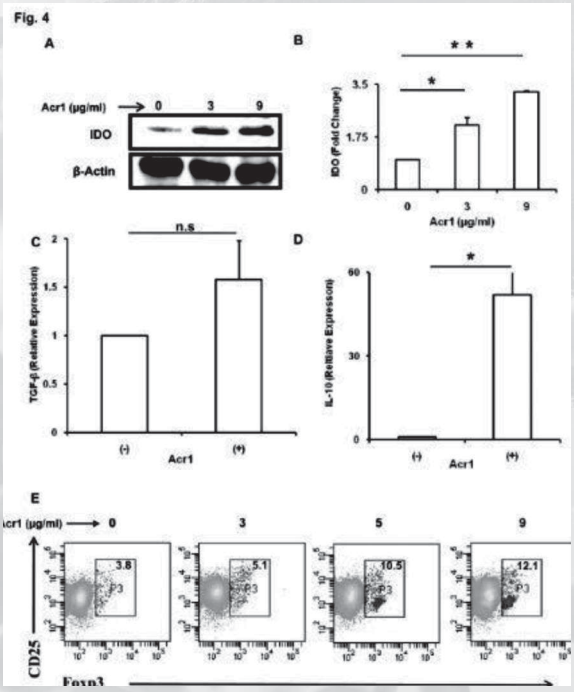


Fig. 4
A
Acr1 (µg/ml)
IDO
β-Actin
B
IDO (Fold Change)
C
TGF-β (Relative Expression)
n.s
D
IL-10 (Relative Expression)
E
CD25
Foxp3
3.8
5.1
10.5
12.1

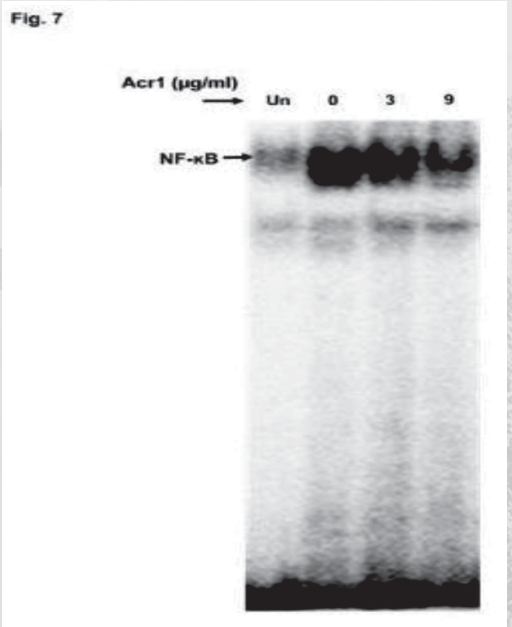


Fig. 7
Acr1 (µg/ml)
Un
0
3
9
NF-κB

ABSTRACT REF.: 013

EFFECT OF BREAST CANCER CELL LINES ON MYELOID MATURATION AND DIFFERENTIATION

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Cancer cells can influence phenotype and function of myeloid cells and facilitate disease progression. The interactions between helper T (Th) cells and myeloid cells at different maturation and differentiation levels are critical for anti-tumor immune responses. In this study, effect of luminal and basal-like breast cancer cells on myeloid differentiation was investigated. Myeloid leukemia cell lines (HL-60, KG-1, U937 and THP-1) served as immature myeloid cells. These cells were co-cultured with basal-like (MDA-MB-231, HCC38) and luminal (MCF-7, BT-474) breast cancer cell lines or cultured in breast cancer cell lines' supernatants. The changes in CD13, CD33, CD11b, CD11c, HLA-DR, CD14, CD34, CD15, CD16, CD103, CD40, CD62L, CD66b, CD83, CCR5 and CCR7 markers was determined by flow cytometry. Reactive oxygen species (ROS) production (via DCFH-DA staining), phagocytosis (via ingestion of fluorescent-labeled opsonized latex beads), and migration (through chemotaxis chamber) were assessed. Th-cell proliferation was determined with CFSE assay.

THP-1 and U937 cells were more affected by breast cancer cells. In co-cultures and in the presence of breast cancer cell supernatants, basal-like breast cancer cells increased expression of CD11b, CD11c, CD14, CD40 and CCR7 on myeloid cells. No difference was observed between luminal and basal-like breast cancer cells influence on ROS production and phagocytosis. Basal-like breast cancer cells enhanced chemotactic capacity of THP-1 cells. Myeloid cells incubated with basal-like breast cancer supernatants tend to decrease Th-cell proliferation.

As compared to luminal breast cancer cells, basal-like breast cancer cells may more efficiently stimulate maturation and differentiation of myeloid cells which eventually modulate Th-cell responses.

Keywords: Breast cancer, myeloid cell, maturation, differentiation, chemotaxis, phagocytosis, ROS, Th-cell

ABSTRACT REF.: 028

IDENTIFICATION BY MICROARRAY ANALYSIS OF INMUNOLOGICAL MOLECULAR MARKERS ASSOCIATED TO CLINICAL RESPONSE IN DC-VACCINATED MELANOMA PATIENTS

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We developed a method for production of therapeutic dendritic-like cells named Tumor Antigen Presenting Cells (TAPCells®) using an allogeneic melanoma-derived cell lysate (TRIMEL) as activation factor and antigen provider. TAPCells-based immunotherapy induced T cell-mediated immune responses and improved long-term survival of stage IV patients (López et al 2009. J. Clin. Oncol.; Aguilera et al 2011, Clin cancer Res.). Importantly, 61% of tested patients (76 out of 124) showed a Delayed Type Hypersensitivity (DTH) reaction against TRIMEL indicating the development of anti-tumor immunological memory. DTH response was associated with prolonged survival of the stage IV responder melanoma patients. Furthermore, we observed that DC-vaccination resulted in the presence of cell subpopulations in peripheral blood leucocytes (PBL) associated to a differential response pattern. These differences encouraged molecular studies aimed to detect differential gene expression patterns induced by TAPCells-vaccine that may allow the identification of molecular prognostic markers. Gene expression was analyzed by microarrays of PBL of 7 responder and 5 non-responder vaccinated patients at different time points. Using computer, mathematical, and molecular biological analysis, new molecular markers were detected with prognostic value that can be useful in the design of improved cancer immunotherapy. Seventeen genes were particularly over expressed in responder patients after vaccination, from which 10 were related to the immune response and 7 related to cell cycle control. These findings were confirmed by Real time RT-PCR. Chemokine receptor CXCR4 and FC-receptor CD32 were confirmed to be overexpressed on cell membrane of T/B lymphocytes and monocytes/macrophage in responder patients. Our study may contribute to the better understanding of clinical immunological responses produced by DC-vaccines and to the finding of molecular markers associated to vaccine-mediated beneficial responses. Supported by FONDECYT 1130320, 1130324, Millennium P09/016-F.

Keywords: melanoma, Dendritic cells, cancer vaccine, tumor immunology

ABSTRACT REF.: 060

EX VIVO EXPANDED AND GENETICALLY MODIFIED NATURAL KILLER CELLS FOR CANCER IMMUNOTHERAPY: FROM PROCESS OPTIMIZATION TO CLINICAL EVALUATION

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The development of any malignancy is under close surveillance by NK cells as well as other members of the immune system. Nevertheless, malignant cells obtain various means to escape from the immune system. It has also been shown that malignant cells not only secrete immunosuppressive factors that inhibit NK cell proliferation but also induce phenotypic aberrations on NK cells via contact-dependent interactions. As a result of all these events, defective immunity secondary to tumor development has been a well-established phenomenon. In order to use autologous NK cells effectively for tumour immunotherapy, a reversal of phenotypic and functional defects is of paramount importance.

This research primarily investigates the feasibility and potential of ex vivo expanded NK cells for cancer immunotherapy. Our results produced a system that has the capacity to expand polyclonal and highly cytotoxic NK cells showing selective anti-tumor activity. Protocols for expansion of these cells from healthy donors and patients with Multiple Myeloma (MM) using current Good Manufacturing Practice (cGMP)-compliant methods have been using automated bioreactors. The elevated cytotoxic activity of expanded NK cells against autologous tumor cells, along

with detailed analysis of phenotypic changes during the expansion process has subsequently shifted attention to the interaction between NK and tumor cells.

Both as a basic method to identify these interactions, and as part of further plans to use genetically retargeted NK cells in cancer immunotherapy, we have investigated methods for efficient lentiviral genetic modification of NK cells. Our studies have resulted in an optimized stimulation and genetic modification process for NK cells that greatly enhances lentiviral gene delivery. Along with NK cell stimulating cytokines, an inhibitor of innate immune receptor signaling that blocks the intracellular detection of viral RNA introduced by the vector has been successfully utilized to enhance gene transfer efficiency, also constituting a proof-of-concept for various other gene therapy approaches. Taken together, the work presented here aims to bring us closer to optimal ex vivo manipulation of NK cells for cancer immunotherapy. Clinical trials with the long-term expanded NK cells as well as further preclinical development of NK cell genetic modification processes are warranted.

Keywords: NK cells, ex vivo expansion, GMP, lentiviral vectors, cancer immunotherapy

ABSTRACT REF.: 059

THE TUMOR SUPPRESSOR P53 INTERACTS WITH PATZ1 EXPRESSED DURING T CELL DEVELOPMENT

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The immune system has evolved to defend multicellular organisms from foreign invaders. The main role of the immune system is to produce a diverse variety of cells, which can specifically recognize and eliminate pathogens. The diversity required for antigen specific T cell receptors (TCR) is achieved by gene rearrangement in the TCR loci during T lymphocyte development. This V(D)J recombination process results in dsDNA breaks and accumulation of the tumor suppressor protein, p53. In this study, we showed the functional interaction of the tumor suppressor p53 and the transcription factor PATZ1 which is expressed during T cell development. We demonstrated that both endogenous and overexpressed PATZ1 interacts with p53 with co-immunoprecipitaiton experiments in HCT116 human colon carcinoma cells. We identified the p53 binding pocket of PATZ1 to be localized in the region between its sixth and seventh zinc finger domains. The interaction between PATZ1 and p53 is specific, as an alternative splice variant, PATZ1-012, which lacks the region between the sixth and seventh zinc fingers, does not bind p53. The site directed point mutations in this binding pocket of PATZ1 impaired the interaction between PATZ1 and p53. We also showed that PATZ1, which can directly bind to p53, could inhibit p53 DNA binding, using DNA pull down experiments. The amount of DNA bound p53 is significantly decreased when co-transfected with PATZ1 but not PATZ1-012, which cannot bind p53. In addition to these, we performed growth curve analysis upon DNA damage inducing drug, doxorubicin treatment in stably PATZ1 expressing HCT116 cells. We demonstrated that PATZ1 overexpressing HCT116 cells significantly have higher IC50 values, which means PATZ1 overexpression makes cells more resistant to doxorubicin induced DNA damage.

The functional interaction between PATZ1 and p53 may be critical for T lymphocyte development, V(D)J recombination, DNA damage and lymphomagenesis.

Keywords: PATZ1, p53, lymphomagenesis, DNA damage, interaction

ABSTRACT REF.: 033

ACYNETOBACTER BAUMANNII HAS GOT AN IMMUNE-EVASION MECHANISM

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Acinetobacter Baumannii is a Gram-negative aerobic, non-motil, nonfermentative pathogen inducing nasocomial and community acquired infections, especially in immunocompromised patients. Currently, incidence of A.Baumannii infections is getting increase due to the rapid development of resistance to antibiotics and persistence in the hospital environment. Several Acinetobacter spp. can colonize human skin and such colonization can serve as a major source for infection, including those of the urinary and respiratory tracts, endocarditis, wounds, septicemia and meningitis. So, the treatment of A.Baumannii infections are becoming increasingly difficult. Unfortunately, the mortality rates of these infections are significantly high. Despite its clinical importance, the innate host defence mechanisms against A.Baumannii have not yet been understood. Recent studies have shown that CD14, TLR-4 signalling and neutrophils, macrophages, NADPH phagocyte oxidase and complement are important in the local bacterial enhancement and dissemination. But, any immune evasion mechanism of A.Baumanni has not yet been known, so far.

METHODS: The mouse macrophage-like cell line J774.1 was obtained from American Type Culture Collection. Clinical isolated and typeable A.Baumannii was used. Acynetobacter Baumannii) were cultured and heat killed at 85oC for 1 h. Heat inactivated and live bacteria were fluorescently labelled using FITC. Fluorescence of extracellular particles was quenched by adding Trypan blue 2%. Bacteria were coated by heat inactivated fetal bovine serum for 30 minutes before phagocytosis assay. Phagocytosis was measured by flow cytometry and IFA. Nitric oxide production was determined by Griess reaction.

RESULTS: We observed that the phagocytosis ratio of heat inactivated A.Baumannii was higher than live bacteria and nitric oxide level produced in response to live A.Baumannii by macrophages was more than heat inactivated one. Antibody dependent cellular phagocytosis contributes to A.Baumannii elimination.

Keywords: acinetobacter, phagocytosis, immune evasion, macrophage

ABSTRACT REF.: 084

FASL-844 T/C POLYMORPHISM: A BIOMARKER OF GOOD PROGNOSIS OF BREAST CANCER IN THE TUNISIAN POPULATION

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The FAS/FASL system plays a crucial role in modulating apoptosis. FASL can trigger cell-death signal cascade by crosslinking with FAS receptor.

The single nucleotide polymorphism, rs763110 (-844 T/C) of the FASL gene, is located within a putative binding motif of CAAT/enhancer-binding protein ß transcription factor. Higher basal expression of FASL is significantly associated with the FASL-844 C allele

compared with the FASL-844 T allele suggesting that the FASL-844 T/C polymorphism may influence FASL expression and FASL-mediated signalling, and ultimately, the susceptibility to cancer. Therefore, we carried out a population-based study to estimate the FASL-844 C allele frequency in our population and to investigate, in a case-control study, the potential association of the FASL-844 T/C polymorphism with the risk and prognosis of breast cancer in Tunisia.

FASL-844 T/C polymorphism was examined in a Tunisian population-based case-control of 438 patients with breast cancer and 332 control subjects using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis.

By using TT genotype as reference, no significant association was found between any genotype and the risk of developing breast cancer. The frequency of the FASL-844 C allele was 46.3% among the cases and 43.7% among the controls. Similarly, by using T allele as reference, this difference was also not statistically significant. We observed FASL-844 CC genotype and FASL-844 C allele were significantly associated with SBR 1-2 tumour grade (OR = 0.42, P = 0.007; OR = 0.65, P = 0.005, respectively). In patients with diagnosis age <=50 years, FASL-844 CC genotype and C allele showed significant associations with T1-T2 clinical tumour size (OR = 0.34, P = 0.01; OR = 0.65, P = 0.02, respectively) and SBR grade 1-2 (OR = 0.41, P = 0.02; OR = 0.62, P = 0.01, respectively). A marginally significant association was also found with negative nodal status (OR = 0.53, P = 0.06; OR = 0.73, P = 0.07, respectively). Thus, the FASL-844 CC genotype and C allele seem to be associated with a good prognosis in patients with diagnosis age <=50 years.

Keywords: Breast cancer, FASL gene, prognosis

ABSTRACT REF.: 016

IMPROVING THE TH1 CELLULAR EFFICACY OF THE LEAD YERSINIA PESTIS RF1-V SUBUNIT VACCINE USING SA-4-1BBL AS A NOVEL ADJUVANT

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The lead candidate plague subunit vaccine is the recombinant fusion protein rF1-V adjuvanted with alum. While alum generates Th2 regulated robust humoral responses, immune protection against Yersinia pestis has been shown to also involve Th1 driven cellular responses. Therefore, the rF1-V-based subunit vaccine may benefit from an adjuvant system that generates a mixed Th1 and humoral immune response. We herein assessed the efficacy of a novel SA-4-1BBL costimulatory molecule as a Th1 adjuvant to improve cellular responses generated by the rF1-V vaccine. SA-4-1BBL as a single adjuvant had better efficacy than alum in generating CD4+ and CD8+ T cells producing TNFa and IFNg cytokines. The combination of SA-4-1BBL with alum further increased this Th1 response as compared with the individual adjuvants. Analysis of the humoral response revealed that SA-4-1BBL as a single adjuvant did not generate a significant anti-rF1-V antibody response, and SA-4-1BBL in combination with alum did not improve antibody titers. However, the combined adjuvants significantly increased the ratio of Th1 regulated IgG2c to the Th2 regulated IgG1. Finally, a single vaccination with rF1-V adjuvanted with SA-4-1BBL + alum had better protective efficacy than vaccines containing individual adjuvants. Taken together, these results demonstrate that SA-4-1BBL improves the protective efficacy of the alum adjuvanted lead rF1-V subunit vaccine by generating a more balanced Th1 cellular and humoral immune response. As such, this adjuvant platform may prove efficacious not only for the rF1-V vaccine but also against other infections that require both cellular and humoral immune responses for protection.

Keywords: Y. pestis, Plague, Vaccine, Alum, SA-4-1BBL

ABSTRACT REF.: 076]

NOVEL FORMULATIONS OF C-DI-GMP ENHANCE ITS IMMUNOSTIMULATORY ACTIVITY

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3´,5´- Cyclic diguanylic acid (c-di-GMP) is a bacteria-derived small cyclic di-nucleotide that functions as the universal bacterial secondary messenger. To date, studies concerning its immunostimulatory effects show that cytosolic sensing of c-di-GMP by the innate immune sensor receptor STING, induces a robust type-I Interferon production in antigen presenting cells, leading to their maturation. However, the chemical structure and the anionic nature of c-di-GMP limit its efficient entry through cellular membranes, requiring its transfection to the cytosol. In this study, we explored the possibility of using two different strategies that would improve the intracellular delivery and/or boost the immunostimulatory activity of c-di-GMP. Specifically, here we present a simple peptide complexation strategy in which complexation of the di-nucleotide with nonaarginine significantly increased cellular uptake and induced higher levels of pro-inflammatory cytokine and Type-I interferon production in mouse splenocytes relative to its native form. As an alternative delivery strategy, we successfully encapsulated c-di-GMP in bacteria derived membrane vesicles (MV) and utilized them as vesicular carriers of the cyclic di-nucleotide. To compare immunostimulatory activities of free versus formulated c-di-GMP in vivo, mice were vaccinated with model antigen OVA in the absence or presence of free c-di-GMP or its formulations. Results showed that, relative to the free ligand, cationic peptide complexation of c-di-GMP or its encapsulation in bacteria-derived membrane vesicles (MVs) boosted antigen-specific immune responses characterized by elevated titers of anti-OVA IgGs and induced a potent protective anti-tumor response to E.G7-OVA tumor challenge. Consistent with our previous observations, ex-vivo re-stimulation of spleen cells harvested from immunized mice with SIINFEKL peptide resulted in 8 to 10 fold increase in IFN-γ production. These results suggest that the immune stimulatory activity of c-di-GMP can be enhanced following nonaarginine complexation or encapsulation in MVs and could contribute to the development of new vaccine adjuvants/immunotherapeutic agents for use in clinic.

Keywords: cylic-di-GMP, cationic peptides, bacterial membrane vesicles, complexation, vaccine adjuvant

ABSTRACT REF.: 036

IRON AND IRON REGULATORY PROTEINS IN MENINGES AND BMDM CULTURES IN NO-DONOR INDUCED MOUSE MODEL OF MIGRAINE

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OBJECTIVE: Inflammation factors, nitric oxide (NO) and related substances are implicated in migraine pathogenesis. There are huge evidences that meningeal cells might be important players in 'neuroinflammatory' component of migraine headache. Oxidative stress, and so NO, as a triggering factor of migraine, may cause to accumulation and especially mislocalization of iron in the cell. The proper homeostasis of cellular iron influx-and efflux-proteins, and proteins involved in iron storage, is important to prevent excess iron overload or iron starvation in cells. In this preliminary study we aimed to show the effects of NO donors on iron related proteins in meningeal and bone marrow derived macrophage (BMDM) cell cultures which might take part in inflammatory process of migraine pathogenesis.

MATERIAL- METHODS: Meningeal cell cultures were prepared from the cerebral cortex of neonatal C57BL/6 mice and BMDM were obtained by differentiating precursor cells from murine bone marrow (n=3). Cells were stimulated with glyceryl trinitrate (GTN) as an NO donor for 24h in both cell cultures. Effects of GTN on iron-efflux protein (ferroportin), iron-influx proteins (Zip14, DMT1 and transferrin receptor 1 (TfR1) and iron-storage protein (ferritin) have been evaluated using qPCR. Iron accumulation in meningeal cells has been evaluated using Perl's histochemistry. Ferritin in meningeal cells have been shown using ferritin immunofluorescence staining.

RESULTS: GTN have stimulated the expression of the ferritin in both cells. Stimulation of BMDM decreased the expression of DMT1 but increased Zip14; despite that in meninges expression of both influx-proteins were increased. TfR1 expression was stimulated in meningeal cells, but inhibited in BMDM. GTN stimulation has caused a huge increase of ferroportin expression in meninges which has been inhibited in BMDM cultures. In meningeal cells, iron accumulation due to GTN in cells was prominent and ferritin accumulations have been verified with immunofluorescence staining.

CONCLUSION: In this preliminary study we have shown that incubation with GTN stimulates iron accumulation in several cell types and triggers the expression of iron regulatory proteins. While ferroportin decrease in macrophages might be related with their phagocytic activity, increase of ferroportin in meningeal cells might cause to an elevation in extracellular iron levels which could be a triggering factor for inflammatory response in meninges during migraine. Based on these results we also have thought that the ferroportin increase could be a survival response of cells without any phagocytic activity to toxic effects of increased iron levels.

Keywords: neuroinflammation, iron, macrophages, meninges, migraine

ABSTRACT REF.: 012

NUMERICAL STATUS OF CD4+CD25+FOXP3+ AND CD8+CD28-REGULATORY T CELLS IN MULTIPLE SCLEROSIS

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OBJECTIVE: Regulatory T cells (Treg), including CD4+CD25+Fox3+ and CD8+CD28- cells play an important role in regulating the balance between immunity and tolerance. Since multiple sclerosis (MS) is an inflammatory autoimmune disease, Tregs are considered to be involved in its pathogenesis. In this study, we investigated the circulatory number of 2-mentioned types of Tregs and also their association with different clinical characteristics in MS patients. METHODS-MATERIALS: 84 patients with MS and 75 normal individuals were studied. The severity of the MS was evaluated through Expanded Disability Status Scale (EDSS) and MRI. The peripheral blood frequency of two different subgroups of Tregs (CD4+ CD25+Foxp3+ and CD8+CD28- cells) were analyzed by Flow cytometry using anti-human antibodies conjugated with CD4+-FITC/CD25+-PE, CD3-PE/CD8a-PE-Cy5/CD28-FITC and intracellular Foxp3-PE-Cy5.

RESULTS: The frequency of CD4+CD25+Foxp3+ cells in MS patients was significantly less than that in healthy controls (P=0.006) (Figure) and in mild forms (CIS, RRMS) less than that in sever forms (PPMS, SPMS, PRMS) (P=0.003) (Table 1). There was not any correlation between the frequency of Tregs and different clinical variables including EDSS scores, number of recurrences, duration of the disease, and duration of the treatment. However, adjusting the effects of different factors, we showed that age affects the frequency of both subset of Tregs (P<0.001) and treatment duration affects the frequency of CD4+CD25+Foxp3+Tregs (P=0.016) (Table 2).

CONCLUSION: Our results showed that the number of CD4+CD25+Foxp3+ cells reduces significantly in multiple sclerosis patients, a concept which probably shows the regulatory role of these cells in MS.

Keywords: Multiple Sclerosis, CD4+CD25+Foxp3+ Regulatory T cells,CD8+CD28-Regulatory T cells

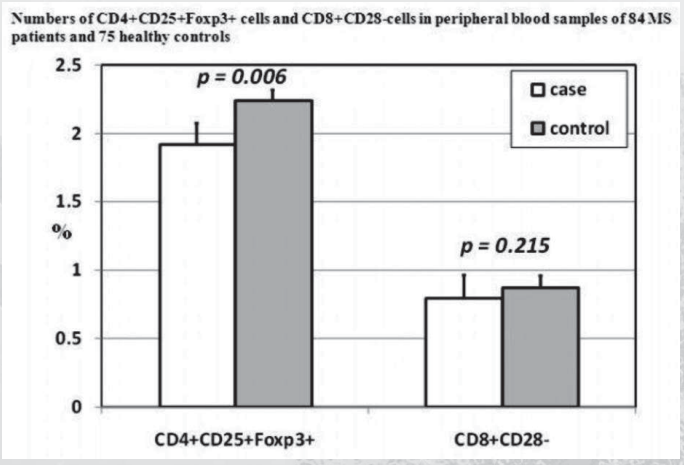

Table 1: Values of Tregs according to different variates in MS patients

		Tregs			
		CD4+CD25+Foxp3+	P	CD8+CD28-	P
EDSS	Mild (0.5-4.5)	1.83±0.7	0.08	0.75±0.4	0.48
	Sever (5-9.5)	2.23±0.7		0.83±0.23	
Type	Mild (CIS, RRMS)	1.86±0.72	0.03	0.8±0.4	0.72
	Sever (PPMS, SPMS, PRMS)	2.25±0.57		0.7±0.26	
sex	Male	2.24±0.8	0.054	0.71±0.34	0.36
	Female	1.84±0.68		0.81±0.38	
Family history	Positive	1.85±0.78	0.764	0.84±0.2	0.323
	Negative	1.92±0.72		0.79±0.39	

Table 2: linear multiple regression analysis evaluating the effect of different factors on the frequency of Tregs in MS patients

	Variables	Coefficients		t	Sig. P	Adjusted R Squared
		B	Std. error			
CD4+CD25+Foxp3+Tregs	Age	0.0489	0.005	9.1	<0.001	0.845
	Sex	0.458	0.252	1.8	0.07	
	Disease duration	-0.041	0.025	-1.63	0.10	
	Treatment duration	0.072	0.029	2.47	0.016	
CD8+CD28-Tregs	Age	0.018	0.002	8.64	<0.001	0.759
	Sex	-0.178	0.134	-1.32	0.18	
	Number of recurrences	0.021	0.012	1.79	0.07	

ABSTRACT REF.: 078

CD4 VERSUS CD8 T CELL LINEAGE FATE DETERMINANT PATZ1 FUNCTIONALLY INTERACTS WITH P53

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BTB/POZ-zinc finger (ZF) transcription factor, PATZ1 (also called MAZR) has 7 zinc-finger motifs in its DNA binding domain and a POZ/BTB protein-interaction domain. PATZ1 is upregulated in colorectal cancer and testicular tumors, while its downregulation in glioma cells sensitizes them to apoptotic stimuli. In another recent study, impaired expression of PATZ1 was found to favor lymphomagenesis by activating the BCL6 pathway. In T lymphocytes, PATZ1 negatively regulates the CD8 gene enhancer. Inactivation of Patz1 gene results in the derepression of the CD4 specific ThPok gene enhancer suggesting that PATZ is also a stage specific controller of thymocyte lineage fate. These findings place PATZ1 in a crucial role during tumor development and also during the CD8 linage differentiation of double positive thymocytes.

We showed that reconstruction of PATZ1 expression in PATZ1-deficient CD8 lymphocytes re-represses ThPOK gene, which is otherwise expressed in CD8 lineage despite it is a CD4 lineage marker. We also identified several amino acid residues in the zinc-finger domain of PATZ1 that are important for the repressor activity.

We have defined another role for PATZ1 as a candidate regulator of the p53 tumor suppressor protein. We found out that upon doxorubicin induced DNA damage, the protein level of PATZ1 decreases while p53 accumulates. We also observed that MEF lacking PATZ1 expression proliferates slower than wild type MEF and consistently has increased doubling time. On the other hand, PATZ1 overexpressing HCT116 cells proliferate faster and have shorter doubling time compared to wild type HCT116 cells. We demonstrated that PATZ1 inhibits the transcription activation function of p53 by luciferase reporter assays. In addition to these, we showed that PATZ1 overexpressing cells have impaired induction of p53 responsive genes, namely p21 and Puma, upon doxorubicin treatment compaired to wild type cells.

We are conducting experiments to deeply investigate the role of PATZ1 on the p53-mediated DNA damage response and p53-dependent tumorigenesis. PATZ1 may be a promising target for the development of new therapeutic strategies against tumor formation.

Keywords: PATZ1, p53, CD8, THPOK, lymphomagenesis

ABSTRACT REF.: 063

ROLE OF CD40L EXPRESSED BY CD8+ T CELLS IN CELLULAR IMMUNITY

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Initiation of effective immunity depends on the communication between innate and adaptive immune cells. CD40-CD40L engagement, one of the key interactions in these processes, is essential for activation of cellular immunity by inducing the maturation of professional antigen-presenting cells to prime an efficient T cell response and as well essential for B cell differentiation and isotype class switching. However, the exact role of CD40L-CD40 axis signaling for the induction and maintenance of immunological memory has remained unclear so far. While initially, CD40L was defined as a highly specific marker for activated CD4+ helper T cells, we have recently shown that CD8+ T cells can also express CD40L upon activation. In this study, we have assessed the role of CD40L expressed by CD8+ memory T cells in infection models such as LCMV and L.m. Our data indicates during viral and bacterial infection a distinct and large subset of memory CD8+ T cells is able to express CD40L. Mice lacking CD40L expression exclusively in CD8+ T cells had no major impairment on primary effector/memory responses in acute LCMV infection. However, upon rechallenge, CD8+ T cells lacking CD40L expression showed higher proliferation with higher IFNg production, indicating a possible breakdown in regulatory mechanism.

Therefore we hypothesize that CD40L expression on memory CD8+ T cells is important for controlling over-activation and immunopathology as a result of secondary activation of memory T cells upon secondary encounter of pathogens.

Keywords: CD40L, CD8+T cell memory, LCMV, infection immunology

POSTERS

AUTOINFLAMMATORY & AUTOIMMUNE DISEASES

P-001 *ABSTRACT REF.: 003*

PECULIARITIES OF T-BET, GATA3, FOXP3 AND RORγT TRANSCRIPTIONS FACTORS EXPRESSION IN GALT OF RATS WITH EXPERIMENTAL DIABETES MELLITUS

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A considerable quantity of researches of patients with a diabetes and its models at animals have shown a close connection of development type 1 diabetes mellitus and changes in an intestine which anticipate appearance of clinical symptoms of disease. Functional polarization of T helpers in gut associated lymphoid tissue (GALT) plays an important role in an induction and progression of T1DM. To study the peculiarities of T-bet, GATA3, FOXP3 and RORγt transcriptions factors expression in gut-associated lymphoid tissues (GALT) of rats with experimental STZ induced diabetes mellitus (EDM). Structure of population of T-bet+, GATA +, FOXP3+ and RORγt+ cells has been studied by the analysis of serial histological sections using the method of indirect immunofluorescense with monoclonal antibodies (Santa Cruz Biotechnology, USA). Images were taken by using a fluorescence microscope PrimoStar (ZEISS, Germany) with a computer-assisted video system AxioCam 5c including the ImageJ software. It has been established that diabetes development was accompanied with increase in quantity of T-bet + and RORγt+ cells in lymphoid structures of ileum, with decrease in total density of GATA3+ and FOXP3+ cells lymphocyte, an insignificant decrease in concentration of T bet and it had no influence on concentration of GATA3 in T-helpers., and also leads mainly to growth of RORγt concentration and decrease of FOXP3 concentration in immunopositive cells. The expression augmentation with these transcription factors in ileum immunopositive cells can influence the differentiation of subsets of T-helpers and their proinflammatory cytokines production, thus acting as one of triggers of diabetes development and progression.

Keywords: diabetes, T helpers, GALT

P-002 *ABSTRACT REF.: 119*

NK AND MDC CELLS CROSSTALKS IN BEHCET'S PATIENTS

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Behcet's disease is a multisystemic aoutoinflammatory disorder characterized mainly by recurrent oral and genital ulcers,uveitis and which frequenly involves the joints, skin, central nervous system (CNS) and gastrointestinal tract. The aetiology of Behcet's disease is not definetely known, but the most widely suggested hypothesis of disease pathogenesis is that a microbial agents or other environmental factors trigger varius immunological abnormailities in genetically susceptible people.

Both dendritic cells (DCs) and Natural killer (NK) cells important effector cells of the innate immune system. During the early stages of innate immune responses NK cells and DCs interact resiprocaly and importance of this interaction higligted many reports. During this interaction, both cells induce other cells functions. DC cells induce NK cells function and in turn NK cell induce modulatory effect on DCs cells. On the other hand this reciprocal interaction bettween NK cells and DCs could regulate DCs matutarion process.

AIM: In our study we investigate the result of crosstalk NK cells and mDC(monocytes Dentritic cells) interactions between Behcet' s patient s and voluntraliy healtyh donors.

METHODS: 6 Behcets Patints and 6 healthy controls inculded study. With ficoll hypeque method we obtained PBMCs and sorted monocytes and NK cells with apropriate antibodies by BD Facs Aria Cell sorter. We obtained mDC as described as before. We coultured NK cells alone or with monocytes and we cultured monocytes alone or with NK cells. We measured expression levels of IFN- gama, IL 10, Nkp30 and Kır3dl 1 for NK cells and we measured mDC cell markers CD14, CD209 and CD83 to observe monocytes maturation process.

RESULTS: There were no significant difference between NK cells parameters (IFN-G,IL-10, Nkp30, and Kır3dl1)and dentritic cells differentation paremeters between Behcet's patients and healthy controls.

Keywords: NK cells, Behcets Disease, monosit derived Dentritic Cells

P-003 *ABSTRACT REF.: 087*

THE RELATION OF TOLL LIKE RECEPTOR 2 GENE WITH ANKYLOSING SPONDILITIS

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The seronegative spondyloarthropathies, are diseases usually diagnosed late and difficult for currative treatment which could be seen in immunity responses directed the "host's own" tissues, triggered by the Toll-like receptors which are the key molecules of the immune system defense. On the cell membrane, Toll like receptors which recognize molecular patterns are found besides Human leukocyte antigen (HLA) that is seen in intensive degree in Ankylosing spondilitis which is a typical diseases of the spondyloarthropathies that effect patient's life-span and quality. In theories such as arthrogenic peptide theory, it has been suggested that HLA molecules resemble some bacteria components, therefore some cytotoxic T cells might trigger the autoimmune tissue damage and inflammation processes with the attachment of these "own-molecules". Considering this condition, it is possible to present false immune responses due to binding exogenic or endogenic molecules by TLRs, if the DNA sequence have any changes while expressing these membran receptors.

In this study we tried to investigate that, if there is a difference for TLR2 (NM_003264) gene sequences, between Ankylosing spondilitis patients and healthy control groups.

We included 50 healthy individuals and 50 AS patients aged between 18-75, whom are diagnosed with regard to AMOR and Modified New York identification criteria, in our study. After taking blood samples, we isolated genomic DNA samples. Polymerase chain reaction was set up with six different primers that are specific for TLR2 third exon. Designed primers spanned a region of 2355 bp. Products are controlled with electrophoresis and purification made by ExoSap-IT kite. Cycle sequencing PCR

was applied with BigDye chemistry method. Then, products are purified with BigDyeXT kite and sequencing was carried out on a ABI-3130 genetic analyser. Differences of results are compared with NCBI GenBank databases.

We found the distribution of Asn199Asn (rs3804099), Ser450Ser (rs3804100), Arg753Gln (rs5743708), Phe707Phe (rs5743705) genotypes in AS patients as; 54%, 1%, 22% and 1% respectively and in the control group as 20, 4%, 12% and 0% respectively.

It is clear that different types of TLRs formed by changing polymorphic regions, would react differently to distinct molecules. In this sense, consideration of our findings, it seems to be a correlation between the TLR2 and Ankylosing spondylitis. We appraised that rs5743705 polymorphism could be a new haplotype SNP for TLR2. However, it should be investigate whether these polymorphisms changed after false signaling or exposure to an infectious agent.

Keywords: oll like receptor, Ankylosing spondilitis, Polymorphisms, TLR2 gene, DNA sequencing.

P-004 *ABSTRACT REF.: 133*

INHIBITORY EFFECTS OF IMMUNOSUPPRESSIVE ODN A151 IN CCL4 INDUCED LIVER FIBROSIS

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Synthetic oligodeoxynucleotides (ODN) expressing telomeric TTAGGG motifs are potent inhibitors of proinflammatory and Th1 biased immune activity elicited by a variety of Toll-Like Receptor (TLR) dependent or independent immune stimuli. Recent studies suggest that activation of TLRs play critical roles in development of liver fibrosis which is an abnormal wound healing response generated against a wide range of insults resulting in the excessive accumulation of extracellular matrix components. In this study we attempted to broaden the application of immunosuppressive ODN A151 by investigating its inhibitory effects on CCl4 induced liver fibrosis model in C57BL/6 mice. In order to induce liver fibrosis mice were injected intraperitoneally with CCl4 (8 µl/g) 8 times with 3 day intervals for a duration of 4 weeks. ODN A151 was administered intraperitoneally (150 µl/mouse) either 1 week or 2 weeks after fibrosis establishment. We tested our hypothesis during and after the induction of experimental fibrosis. We analyzed the expression levels of AST, ALT, α-SMA, TLRs, IL6 and IL12 both at mRNA and protein levels in the absence and presence of suppressive ODN A151. Sera collected either control or CCl4 injected mice and liver fibrosis establishment were assessed by elevated serum AST and ALT levels at 4 weeks. In addition, CCl4 treatment significantly enhanced protein levels of α-SMA in fibrotic liver. At the end of 6 weeks, mice sacrificed and liver and spleen tissues were obtained. Our data suggested that CCl4 treatment significantly upregulated TLR2 and TLR4 mRNA levels in fibrotic liver tissues which is an indicator of their roles in liver fibrosis establishment. Furthermore, our data revealed that ODN A151 significantly decreased αSMA protein levels in fibrotic liver. Moreover, the expression of TLRs 1-9 were also down regulated significantly after systemic administration of ODN A151. Of note, among down-regulated TLRs, TLR2, TLR4, TLR6 and TLR9 were the most strongly suppressed receptors. Ex-vivo immune stimulation assays revealed that splenocytes isolated from ODN A151 injected mice exhibited increased IL6 and decreased IL12 levels upon TLR ligand stimulation compared to CCl4 only injected mice. In conclusion, administration of suppressive ODN A151 contributed to the regression of liver fibrosis. Our data suggested that targeting TLRs with suppressive ODN A151 may be a promising strategy to reverse the progress of this destructive process and may offer an alternative approach to control the progress of liver fibrosis into cirrhosis, thus decrease the need for liver transplantation.

Keywords: Liver Fibrosis, CCl4, Immunosuppressive ODN A151, TLRs,

P-005 *ABSTRACT REF.: 113*

HOW COULD BE USED THE AUTOANTIBODIES AGAINST ANTI-TNF AGENTS IN CLINICAL PRACTICE? TWO YEARS FOLLOW-UP STUDY

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BACKGROUND: Immonogenicity is one of the major causes for non-responding to anti-TNF treatment, and recently different anti-drug antibody (ADA) ratios for different diseases were reported. Therefore there is not generally recommended guidelines to investigate the immunogenicity in inflmmatory diseases. The ADAs, drug levels and the clinical progress of patients with ADA after 2 years follow-up period were investigated.

METHOD: Serum levels of anti-infliximab, adalimumab and etanercept ADAs and drug levels were obtained by ELISA in ankylosing spondylitis (AS) patients (n=72, mean age:40.3 ± 10.0) and rheumatoid arthritis (RA) patients (n=24, mean age:46.2 ± 11.5).

RESULTS: Five of the infliximab treatment received AS patients (n=46) had ADA positivity, one of them had infusion reaction. The other four patients were good responder but one of them had resistance against infliximab after 11 months. The infliximab levels of ADA positive patients were undetectable. None of AS patients (n=26) under etanercept therapy had ADA. In adalimumab receiving patients the drug levels of ADA carrying patients were different, also these antibodies were non-neutralizing Ab.The statistically significant median levels of antibody detecting time was found as 38 months in infliximab receiving patients and 6 months in adalimumab receiving AS patients (p=0.05). RA patients (n=12) receiving infliximab therapy, ADA detected in 2 of them and one of them had a good response. The drug levels of ADA carrying patients were $p<0.05$, also these autoantibodies were neutralizing Ab. RA patients (n=15) receiving etanercept, no ADA has been detected. TNF response in RA patients, there was no significant difference when compared between infliximab&etanercept and infliximab&adalimumab therapy (p=0.93 and p=0.65, respectively). The ADA levels between adalimumab and infliximab there was also no significant difference (p=0.603) obtained. The median levels of antibody detecting time was found 12 months in infliximab receiving RA patients and 7.5 months in adalimumab receiving RA patients and the difference was not significant (p=0.121).

CONCLUSION: The ADA and drug level analysis could be useful in decision-making about the choice of treatment for non-responder patients. It is needed the standardization of the methods for analyzing immunogenicity.

Keywords: anti TNF therapy, ADA, ankylosing spondylitis, rheumatoid arthritis

P-006 *ABSTRACT REF.: 109*

A RETROSPECTIVE EVALUATION OF ANTINUCLEAR ANTIBODY POSITIVITY IN A UNIVERSITY HOSPITAL

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Patients with suspected autoimmune disease were screened for the presence of antinuclear antibodies (ANA) using an indirect fluorescent assay (IFA).

A total of 5427 patients with suspected autoimmune disease were screened retrospectively for the presence of autoantibodies by the Medical Microbiology Laboratories of Sakarya University Educational and Research Hospital between May 2012 and March 2014. Samples were screened for reactivity against human epithelial cancer (HEp) 2010 cells, and evaluated using indirect fluorescence microscopy.

Of the ten clinics referring patients for evaluation, Rheumatology (35.1%), Physical Medicine and Rehabilitation (17.4%), Internal Medicine (13.2%), Dermatology (12.1%) and Neurology (7.4%) were the most common. ANAs were detected in 29.9% of patients (1625), and classified based upon patterns of reactivity. The largest percentage of ANA positive patients were associated with the Rheumatology clinic (31.2%; 507/1625), followed by Physical Medicine and Rehabilitation (18.1%; 294/1625). Among ANA positive patients, the most common fluorescence patterns were nuclear (48.7%), nucleoli (19.3%), mitotic (17.4%) and cytoplasmic (14.6%).

ANA-positivity rates identified in this study were higher than other published studies. Follow up studies are planned with each of the relevant clinics, particularly regarding analysis of lower titer-positive patients. Standardization is necessary for ANA data placed in the literature, and optimization of results is being performed to improve the positive and negative predictive values of the test.

Keywords: Indirect immune fluorescence test, antinuclear antibodies, autoimmunity

P-007 *ABSTRACT REF.: 132*

THE INVESTIGATION OF KILLER CELL IMMUNOGLOBULIN LIKE RECEPTOR (KIR) GENOTYPING IN PATIENTS WITH SYSTEMIC LUPUS ERYTEMATOSUS AND SYSTEMIC SCLEROSIS

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Systemic lupus erythematosus (SLE) is an autoimmune disease characterised by the production of autoantibodies and the involvement of multiple organ systems. The etiopathogenesis of SLE is unknown. Systemic sclerosis (SSc) is another autoimmune disease that effects mainly connective tissue. Its pathogenesis is unknown too.

We will aim to analyse the role of killer cell immunoglobulin-like receptors (KIRs) genotypes and their existence with the respective HLA ligands in the pathogenesis of SLE and SSc. We examined the presence/absence of KIR2DL1, 2DL2, 2DL3, 2DL4, 2DL5A, 2DL5B, 2DS1, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DL1, 3DL2, 3DL3, 3DS1, 2DP1, 3DP1 and their known HLA ligands in 45 SLE, 25 SSc and 40 healhty controls.

The KIR2DL5B (46.8% vs 25%, P = 0.036; OR = 2.6, 95% CI 1.05-6.6) and KIR2DS3 (42.6% vs 22.5%, P = 0.048; OR = 2.5, 95% CI 0.99-6.5) gene phenotype frequencies were found to be significantly increased in SLE patients compared to healthy controls. KIR2DS3 gene frequency was observed significantly increased in SSc patients too (48% vs 22.5%, P = 0.032; OR = 3.1, 95% CI 1.08-9.36). We did not find any associations of other observed KIR genes between the patients groups and controls. No significant difference was observed for KIR ligand between the patient groups and controls as well.

KIR2DL5B and KIR2DS3 genes may have an role in autoimmune mechanisms. We are investigating this KIR genes epigenetic variations between the study groups. This study was supported by TUBITAK (111S153).

Keywords: killer cell immunoglobulin like receptor, Systemic Lupus Erytematosus, Systemic Sclerosis

P-008 *ABSTRACT REF.: 099*

ADAMTS1 IS MATRIX PROTEINASE INDUCED IN PREECLAMPSIA

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Preeclampsia (PE) is a pregnancy-specific disease and it is characterized by high blood pressure, endothelial dysfunctions, high protein level in urine and placentation defects in the pregnancy. During the remodeling of the placenta, due to PE, cytotrophoblast endovascular invasion can lead uteroplecental circulation. Proangiogenic and antiangiogenic factor balance is mainly depends on the severity of the disease. Extracellular matrix (ECM) is important for placentation and ECM exert its major role for remodeling processes. Recently, newly identified new proteinases family, namely, ADAMTSs, involving in human endometrial stromal cells during decidualization, pregnancy and premature ovarian failure. Interestingly, recently ADAMTS16 was identified as blood pressure controlling candidate gene and ADAMTS1 discovered that it is related to inflammation. However, currently, pathogenesis of preeclampsia and any ADAMTS connection with PE are not well known. To found any potential connection between PE and ADAMTS1, we design our study and we hypothesied that disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS1) may be involved in the pathogenesis of PE. To do this, we investigated potential level of the ADAMTS enzymes in preeclampsia. Western Blot analysis is performed by using control and PE patients. Western Blot analysis demonstrated that ADAMTS1 protein in the PE placenta tissues, was detected and stimulated in the PE diseased tissues. We found 5 fold increase.ADAMTS1 might have a role in the pathogenesis of PE. So, the correlation of these enzymes in disease pathogenesis is worth to investigate.

Keywords: Preeclampsia, ADAMTS1, pregnancy-specific disease, inflammation

P-009 *[ABSTRACT REF.: 139*

THE RELATION OF TOLL LIKE RECEPTOR 2 GENE WITH ANKYLOSING SPONDILITIS

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The seronegative spondyloarthropathies, are diseases usually diagnosed late and difficult for currative treatment which could be seen in immunity responses directed the "host's own" tissues, triggered by the Toll-like receptors which are the key molecules of the immune system defense. On the cell membrane, Toll like receptors which recognize molecular patterns are found besides Human leukocyte antigen (HLA) that is seen in intensive degree in Ankylosing spondilitis which is a typical diseases of the spondyloarthropathies that effect patient's life-span and quality. In theories such as arthrogenic peptide theory, it has been suggested that HLA molecules resemble some bacteria components, therefore some cytotoxic T cells might trigger the autoimmune tissue damage and inflammation processes with the attachment of these "own-molecules". Considering this condition, it is possible to present false immune responses due to binding exogenic or endogenic molecules by TLRs, if the DNA sequence have any changes while expressing these membran receptors.

In this study we tried to investigate that, if there is a difference for TLR2 (NM_003264) gene sequences, between Ankylosing spondilitis patients and healthy control groups. We included 50 healthy individuals and 50 AS patients aged between 18-75, whom are diagnosed with regard to AMOR and Modified New York identification criteria, in our study. After taking blood samples, we isolated genomic DNA samples. Polymerase chain reaction was set up with six different primers that are specific for TLR2 third exon. Designed primers spanned a region of 2355 bp. Products are controlled with electrophoresis and purification made by ExoSap-IT kite. Cycle sequencing PCR was applied with BigDye chemistry method. Then, products are purified with BigDyeXT kite and sequencing was carried out on a ABI-3130 genetic analyser. Differences of results are compared with NCBI GenBank databases.

We found the distribution of Asn199Asn (rs3804099), Ser450Ser (rs3804100), Arg753Gln (rs5743708), Phe707Phe (rs5743705) genotypes in AS patients as; 54%, 1%, 22% and 1% respectively and in the control group as 20, 4%, 12% and 0% respectively. It is clear that different types of TLRs formed by changing polymorphic regions, would react differently to distinct molecules. In this sense, consideration of our findings, it seems to be a correlation between the TLR2 and Ankylosing spondylitis. We appraised that rs5743705 polymorphism could be a new haplotype SNP for TLR2. However, it should be investigate whether these polymorphisms changed after false signaling or exposure to an infectious agent.

Keywords: oll like receptor, Ankylosing spondilitis, Polymorphisms, TLR2 gene, DNA sequencing.

P-010 *ABSTRACT REF.: 138*

A RETROSPECTIVE EVALUATION OF LABORATORY CHARACTERISTICS OF PATIENTS WITH SUSPECTED RHEUMATOID ARTHRITIS

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OBJECTIVES: The aim of this study was to characterized a population of patients with rheumatological signs and symptoms according to laboratory tests, including evidence of inflammatory activity by erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP); presence of autoantibody rheumatoid factor (RF) and cyclic citrullinated peptide (CCP) antibody.

METHODS: In the study, the data of 1006 patients (811 female) who were referred to Physical Medicine and Rehabilitation (382 patient) and Rheumatology (624 patient) clinics with rheumatic complaints and findings between November 2011 and February 2014 was evaluated retrospectively. CCP, RF, ESR, CRP results were evaluated simultaneously. The ESR was determined by standard methods, while the RF and CRP were measured by immunonephelometry. Anti-CCP measurements were calculated using Anti-CCP reagent ELISA test kit (Abbott Diagnostics Inc, USA) in accordance with the manufacturer's protocol.

RESULTS: The results for anti-CCP, RF, CRP and ESR were positive in 423 (42%), 439 (43,7%), 329 (32,7%) and 328 (32,6%) patients respectively. Anti-CCP antibodies were positive in 313 patients RF, in 316 patients ESR and in 327 patients CRP were positive.

CONCLUSION: Anti-CCP is seemed to be superior to other serological markers in the diagnosis of early RA. The anti-CCP and RF combination provided the best test sensitivity.

Keywords: Anti-cyclic citrullinated peptide; rheumatoid factor; suspected rheumatoid arthritis.

P-011 *[ABSTRACT REF.: 097*

INVESTIGATION OF THE ROLE OF COPY NUMBER VARIATION IN THE SUSCEPTIBILITY TO SYSTEMIC LUPUS ERYTHEMATOSUS

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Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease characterised by immune complex deposition and production of autoantibodies against nuclear antigens (ANA). The aetiology of SLE is complex; however, twin and genome-wide association (GWA) studies clearly indicate that SLE has a strong genetic component. Most recently, Copy Number Variations (CNVs) have been shown to be ubiquitously present in unrelated healthy subjects and contribute substantially to phe-

notypic variation in the human population. Furthermore, there is increasing evidence that CNVs contribute to the pathogenesis in autoimmune diseases such as Type 1 diabetes, psiorasis and SLE.

In order to investigate SLE-associated genes with CNVs, Affymetrix Human Genome-wide SNP array 5.0 analysis was performed across 66 samples from our APOSLE (Australian Point Mutations in SLE) cohort. 24, 094 CNVs were identified, however, low log2R values suggested many were likely to be false positive CNVs. For this reason, we used a filtering approach to identify positive CNVs reducing the number to 981. Out of these 981 CNVs, we selected 13 CNVs based on the selection criteria list developed to be able to detect the CNV most likely associated with SLE disease. We used qRT-PCR to verify gene CN in firstly the individuals of interest and then the larger human DNA cohorts. CNVs in the CFHR1/3, RASGRP3, were verified as true positive results. We screened two different human cohorts, the APOSLE and European, for the presence or absence of these CNVs, to determine if these CNVs showed a different trend in SLE patients (divided into those with and without nephritis) compared to healthy control subjects. Using this method, we identified two genes, CFHR1/3 and RASGRP3, where loss of CN was more frequently found in SLE patients without nephritis. Thus the results presented in this thesis suggest CNVs in the CFHR1/3, RASGRP3 may act as a protective factor against the development of nephritis in patients with SLE.

Keywords: Copy number variation, Systemic lupus erythematosus

P-012 *ABSTRACT REF.: 025*

EXPLORING THE ASSOCIATION BETWEEN INTERLEUKIN 18 PROMOTER POLYMORPHISMS AND VITILIGO

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Vitiligo is a common pigmentary disorder caused by the destruction of functional melanocytes. Its immunopathogenesis is not completely understood, but inflammatory alterations in the skin microenvironment, and particularly increased expression of the cytokines, are thought to be essential regulators of melanocyte dysfunction and death. Interleukin 18 (IL-18) is a cytokine that plays an important role in the Th1 response, by its ability to induce IFN-γ production in T cells and natural killer cells. The IL-18 promoter is highly polymorphic and the -137 G/C (rs187238) and -607 C/A (rs1946518) SNPs in the promoter affects its promoter activity and IL-18 production was demonstrated. To our knowledge, there is no study that has investigated association between vitiligo/active vitiligo and IL18 gene polymorphisms. We investigated the frequencies of the two above mentioned IL18 promoter alterations in vitiligo/active vitiligo patients and control subjects to determine whether these variants might represent susceptibility factors for vitiligo. IL18 promotor polymorphisms were examined by PCR-RFLP method. There were no significant differences in the genotype and allele frequency of IL18 rs187238 and rs1946518 SNPs when we compared vitiligo/active vitiligo patients to healthy subjects. The frequency of the CC genotype of IL18 rs187238 tended to decrease in vitiligo patients compared to healthy subjects, but was not statistically significant (p=0.213). In haplotype analysis of two SNPs in the IL-18 gene also did not reach the statistical significance (p=0.715). In conclusion, our results suggest that IL18 gene polymorphisms were not play a key role in the pathogenesis of vitiligo/active vitiligo.

Keywords: Vitiligo, IL18, Polymorphism

P-013 *ABSTRACT REF.: 043*

GENETIC VARIANTS OF DEATH RECEPTOR 4 (DR4) AND SERUM LEVEL OF TRAIL IN VITILIGO

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Vitiligo (as known leukoderma) is a chronic skin disease that causes loss of pigment due to destruction of melanocytes, resulting in irregular pale patches of skin. Vitiligo is a polygenic, multifactorial disease. Vitiligo is associated with autoimmunity with an unknown genetic etiology. It has known that, apoptotic mechanisms effect to Vitiligo pathogenesis. The aim of our study was to further investigate the relationship between genetic variants of Death receptor 4 (DR4) and serum levels of TNF-related apoptosis-inducing ligand (TRAIL) in vitiligo. Seventy-nine patients with vitiligo and unrelated healthy 84 controls were included in the study. Alleles and genotypes of DR4 gene polymorphisms (rs6557634, rs20575 and rs20576) were determined with PCR and RFLP methods. Also, TRAIL levels were measured in serum. We have shown that, TRAIL levels in vitiligo patients were significantly higher than healthy controls ($p < 0.05$). However, it has not found any relationship between rs6557634, rs20575 and rs20576 polymorphism of DR4 gene and vitiligo patients ($p > 0.05$).

The present results indicate for the first time TRAIL levels increase in vitiligo patients because of the role of apoptotic mechanism. Also, it has known that one of the receptor of TRAIL (DR4) gene polymorphisms (rs6557634, rs20575 and rs20576) is not effective for vitiligo disease.

Keywords: Vitiligo, Trail, Polymorphism

P-014 *ABSTRACT REF.: 044*

ASSOCIATIONS OF TLR2 AND TLR4 GENE POLYMORPHISMS WITH PSORIASIS DISEASE

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Psoriasis is a common chronic and recurrent disease which affects the joints together with the skin. Although the etiopathogenesis of psoriasis has not precisely determined, the most supported mechanism is increases of inflammation in the skin. The recently described family of toll like receptors (TLRs) play a critical role in host immunity by mediating inflammatory reactions against a wide range of pathogens. In our study, it is aimed to investigate the possible relations of some TLR gene polymorphisms with psoriasis which have not been investigated yet. 100 patients diagnosed with Psoriasis and 173 healthy controls were

enrolled in the study. TLR2 gene Arg677Trp, Arg753Gln, -196-174 del and TLR4 gene Asp299Gly, Thr399Ile polymorphisms were determined by PCR-RFLP method. Patient and control groups were compared for TLR2 and TLR4 gene polymorphisms. In this study, it was found that there is a statistically significant relationship between psoriasis and GA genotype and A allele of TLR2 Arg753Gln polymorphism. Furthermore, when patient and control groups were compared for TLR2 -196-174 del gene polymorphism, ins/del genotype was determined to have a protective effect. Allele and genotype distribution of TLR2 gene Arg677Trp, TLR4 gene Asp299Gly and Thr399Ile gene were not different in patients and control groups. We suggest that variant alleles in the TLR2 gene may play an important role in the molecular etiopathogenesis of psoriasis. The future studies need to investigate TLR gene polymorphisms on the larger scale in order to understand etiopathogenesis, development, and the prognosis of psoriasis.

Keywords: Psoriasis, TLR2, TLR4, polymorphism

P-015 *ABSTRACT REF.: 062*

A FUNCTIONALLY SIGNIFICANT POLYMORPHISM IN IL-17 GENE IS ASSOCIATED WITH PSORIASIS

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Psoriasis is a complex chronic inflammatory disease, developing by immune-mediated mechanisms with an average of 2% incidence. Although the primary cause of the etiology has not been fully disclosed its thought to be multifactorial and multigenic. In this study, in psoriasis patients, our aim was to determine the frequency of IL-17F (Glu126Gly, His161Arg) and IL-17A (G197A) gene polymorphisms and its effects on psoriasis development risk, age of onset, nail involvement, type of psoriasis and psoriatic arthritis.

The study includes 100 psoriasis dermatologic clinic outpatients and 100 healthy volunteers who have no diagnosis either psoriasis or autoimmune diseases at themselves and their family. PCR (Polymerase chain reaction) method was used to determine IL-17F (Glu126Gly, His161Arg) and IL-17A (G197A) gene polymorphisms in the patient and control groups.

There was no significant difference between in terms of IL-17F (Glu126Gly) and IL-17 (G197A) gene polymorphisms between psoriasis patients and healthy controls. IL-17F His161Arg CT genotype was found to be significantly higher in the control group compared to patients with psoriasis (p=0.003). In addition, IL17 gene polymorphisms were compared between groups of patients according to the character. In psoriatic arthritis patients IL-17 (G197A) GG genotype (p=0.028) was found to be significantly higher than psoriasis patients. Patients without nail involvement compared to patients with nail involvement and IL-17F (Glu126Gly) AG genotype (p=0.008) were found to be significantly increased. Three haplotype frequencies that belong to these polymorphisms were evaluated in the patient and control groups. ACA haplotype was found to be significantly higher in the control group.

In conclusion, IL-17F His161Arg CT genotype may be protective in terms of the risk of developing psoriasis. In addition, IL-17A (G197A) GG genotype may be a risk factor for the development of psoriatic arthritis.

Keywords: Psoriasis, Polymorphism, IL-17A, IL-17F

P-016 *ABSTRACT REF.: 089*

IL17 AND IL2 GENE POLYMORPHISMS IN PATIENTS WITH OSTEOARTHRITIS OF THE HIP

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Osteoarthritis (OA) which causes physical failure in adult population is a chronic degenerative joint disease. The molecular pathogenesis of the disease is not been fully understood yet. However, it is known that cytokines, growth factors, and various inflammatory mediators implicated in pathogenesis of OA. The balance between the production of pro- and antiinflammatory cytokines and/or their functional receptors creating a complex cytokine network influences immunity and inflammation. This balance may be shifted toward the production of proinflammatory cytokines such as IL-2, TNF-α for too long, leads to degradation of the extracellular matrix (ECM) proteins of cartilage such as type II collagen (CII), proteoglycans, and hyaluronic acid. Also recent data have indicated that an IL17 producing Th cell subset is responsible of the bone damage observed in autoimmune arthritis. Interindividual differences in cytokine profiles appear to be due, at least in part, functional polymorphism of cytokine gene may play a role in the incidence and the progression of OA. We aimed with this study, better understanding of the immunopathogenesis of this disease which encountered quite frequently in the society and reduce the quality of life. We investigated the genetic variations of IL17 and IL2 in 72 patients with OA of the hip and 101 healthy subjects as controls. PCR-RFLP method was used to identify the genotypes of IL17F His161Arg, IL17F Glu126Gly, IL17A G197A and IL2G330T polymorphisms. For the IL17F His161Arg polymorphism the risk of OA in carriers with a C allele was statistically higher compared to that of carriers with the T allele (OR:0,33 95%-CI: 0,134-0,848;p:0,019). Haplotypes belonging to IL17F His161Arg ve IL17F Glu126Gly polymorphisms were compared with patients and control group; T/G haplotype was found to increase relative risk of OA 2.4 times (OR 2,4 95% CI 1,154-4,994; p=0,008). However, we could not find any relationship between OA and the IL17A and IL2 polymorphisms. In conclusion our data suggests a protective effect of IL17F His161Arg C allele for developing OA. In addition, T/G haplotype might be a risk factor for the development of OA.

Keywords: Osteoarthritis, IL17, IL2, polymorphism

P-017 *ABSTRACT REF.: 096*

A STUDY OF THE IMPACT OF TLR2 AND TLR4 GENE POLYMORPHISMS IN ATHEROSCLEROTIC STROKE

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Stroke is the second most common cause of death and the leading cause of disability worldwide. About half of all strokes are caused by atherosclerosis. In the past, atherosclerosis was characterized by lipid deposition in the vessel wall. Today's picture is far more complex. Atherosclerosis is considered a chronic inflammatory disease that results in the formation of plaques in large and middle sized arteries. Inflammatory processes or immune responses are involved in the formation of atherosclerosis. Toll-like receptors (TLRs) are pivotal components of the innate immune response. There has been accumulating evidence for the involvement of TLR2 and TLR4 in the pathogenesis of atherosclerosis. The aim of the study is to investigate relationship between TLR2 gene Arg677Trp, Arg753Gln, -196-174 del and TLR4 gene Asp299Gly, Thr399Ile gene polymorphisms and atherosclerotic stroke. TLR2 gene Arg677Trp, Arg753Gln, -196-174 del and TLR4 gene Asp-299Gly, Thr399Ile gene polymorphisms were determined by using PCR-RFLP method in 130 large vessel atherosclerotic stroke patients and 75 healthy control subjects. This study showed that for TLR2 Arg753Gln polymorphism, in the patient group, the frequency of the A allele (4.7%) was higher in comparison with that of the control group (0.5%) and it was determined that A allele was associated with 10.4 fold increased risk of stroke (OR=10.872, 95%CI: 2.452-43.988). Additionally for TLR4 Thr399Ile polymorphism, T allele was associated with 8.9 fold increased risk (OR=8.909, 95% CI: 2.706-29.334) atherosclerotic stroke. In conclusion, our results suggest that genetic polymorphisms in the innate immune system genes may contribute to interindividual differences in the development of atherosclerotic stroke.

Keywords: Stroke, Polymorphism, TLR2, TLR4, Toll-like Receptor

P-018 *ABSTRACT REF.: 038*

EVALUATION OF AUTOANTIBODIES ASSOCIATED WITH AUTOIMMUNE HEPATITIS IN ELDERLY PATIENTS

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AIM: Elderly patients have significantly decreased reserve functions of various organs, reducing their tolerability to treatments for liver diseases. Changes in immune system by aging alters the pathogenesis of viral hepatitis and autoimmune liver diseases, as well as the development of hepatocellular carcinoma. In this study, we aim to evaluate autoantibodies associated with autoimmune hepatitis in elderly patients.

METHOD: Patients aged 65 and older who attended to the outpatient clinic in our hospital were included in this study. Autoantibodies representing autoimmune hepatobiliary diseases such as smooth muscle antibodies (ASMA), antinuclear antibodies (ANA), antimitochondrial antibodies (AMA), liver kidney microsome antibodies (LKM) and gastric parietal cell antibodies (GPC) of 135 patients were evaluated using immunofluorescence.

RESULTS: Of the patients included in the study, 78 were female (57.8%) and mean age was 72.8 (65-89). Seropositivity rate for at least one antibody was 42.2% and 37 (68.4%) were female. Of 135 patients, 33 (24.4%) were positive for ANA, 13 (9.6%) were positive for GPC, 9 (6.7%) were positive for ASMA and 2 (1.5%) were positive for AMA. No positivity was detected for LKM. The most highest positivity rate was detected for ANA and 63.6% were female. The titres for ANA was 1/80 in 12, 1/160 in 7, 1/320 in 8, 1/640 in 3 and 1/1280 in 3 patients.

CONCLUSION: Autoimmune hepatitis affects mainly females and is associated with a number of distinct circulating autoantibodies. In the present study, 68.4% of the elderly patients were female and we observed that ANA was the most common autoantibody. Autoimmune hepatitis should be also considered as a diagnosis for elderly patients because it may be misdiagnosed in the early stage due to the fact that elderly patients may not exhibit any symptoms.

Keywords: Elderly, autoantibodies, autoimmune hepatitis

P-019 *ABSTRACT REF.: 039*

HBSAG SEROPOSITIVITY IN THE RHEUMATOID ARTHRITIS PATIENTS

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AIM: Rheumatoid arthritis (RA) is a progressive and destructive inflammatory disease of the joints. It is the most common chronic inflammatory joint disease in adults. Hepatitis B virus infection is also a health problem in the world. Turkey is intermadiate endemicity region for hepatitis B. The hepatitis B surface antigen (HBsAg) carrier rate is 3.9-12.5 %. Aim of this study is to determine prevalence and screening of HBsAg seropositivity in rheumatoid arthritis patients.

METHOD: This retrospective study was based on a review of the medical records of rheumatoid arthritis patients examined between January 2011 and December 2013 at the rheumatology outpatient clinic of the Izmir Tepecik Education and Research Hospital.

RESULTS: Total of 92 rheumatoid arthritis patients were tested for HBsAg. Mean age was 52.2 (range, 21-77 years) 82.7% were women. Tests were positive in 4 for a seroprevalence of 8.6%. Seventy five percent of positive patients were women with a mean age of 55.3 years (49, 56 and 61 years respectively).

CONCLUSION: HBsAg seroprevalence in our patients with RA was 8.6% similar to the general Turkish population (3.9-12.5%). In our opinion, it is important to screen RA patients for HBsAg before the immunosupressive therapy.

Keywords: Rheumatoid arthritis, HBsAg, Seroprevalance

P-020 *ABSTRACT REF.: 101*

ADAMTS1 PROTEIN ANALYSIS IN HYPERGLYCEMIC RAT LIVER

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Type1 Diabetes(T1D) is autoimmune, metabolic disorder results in chronic hyperglysemia and complications. Hormonal replacement therapy doesn't suffice actual in vivo response of functional pancreatic islets to variying glucose concentration.

Including liver, all tissue cells are surrounded by Extracellular Matrixs(ECM) which takes active role in regulation of cellular activities: scaffolding, differentiation, cell death. Members of ADAMTS family are involved in vital processes: proteolytic modifications of ECM, induced inflammatory conditions, etc.

We've designed series of experiments to deduce ADAMTS profile in T1D and began with comparison of ADAMTS1 proteins in organs of diabetic and non-diabetic rats. Major purpose of the experiments is to investigate the leading cause of diabetes and/or effected pathways by diabetes. STZ(45mg/kg) injected to wistar rats(8-12 week, n=5). Rats of which blood glucose level measured above 250 mg/dl are accepted as diabetic group. Untreated healthy rats used as control group. Rats undergone liver removal. Liver tissue was homogenized, liver proteins were isolated and quantified spectrophotometrically. ADAMTS1 and β-actin proteins were investigated by Western Blotting.

When compared liver proteins acquired from diabetic and non-diabetic rats, we observed significant increase in active form of ADAMTS1 protein and nonsignificant increase in ADAMTS1 protein level when hyperglycemic group was compared to normoglycemic group.

This outcome indicate the fact that ADAMTS1, an anti-angiogenic molecule, takes active role in physiopathology of diabetes. With further investigations, we will search for the role of the proteases in pathogenesis of diabetes.

Keywords: ADAMTS, Proteinaz, Diabetes Marker, Liver, Western Blotting

EMERGING ISSUES IN THE ADAPTIVE IMMUNE SYSTEM

P-021 *ABSTRACT REF.: 103*

INTERFERON-GAMMA INDEPENDENT RESPONSE OF U937 CELLS TO HIGH GLUCOSE

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Glucose is a critical component in the pro-inflammatory response of macrophages. Increased utilization of glucose induces a reactive oxygen species -driven pro-inflammatory phenotype in macrophages which may play an integral role in the promotion of insulin resistance in diabetes. High glucose increases lipid peroxidation, IL-6, IL-8, MCP-1, and TNF-alpha secretion from cultured U937 monocytes. However, it remains unclear how exposure to high glucose might render the macrophages more sensitive to oxidative stress and chronic immune activation. In this study we have tested whether the high concentration of glucose induces DNA damage, neopterin synthesis as a chronic immune activation marker and tryptophan degradation by U937 monocytic cells without IFN-gamma stimulation. The human U937 monocytic cells were exposed to glucose alone and glucose plus 10, 40, 60 uU/ml insulin in seven different concentrations at three different time-periods. To determine toxicity levels of glucose and glucose plus insulin, cell viability was estimated by MTT test. Average value of each point was calculated by measuring eight samples. In order to indicate the DNA damage, Comet assay was used. Neopterin levels were measured by ELISA, kynurenine and tryptophan concentrations were measured with high performance liquid chromatography. U937 cells in the presence of high glucose (>200 mg/dl), showed a significant DNA damage which confirmed genotoxicity. While the increasing glucose concentrations with or without insulin showed higher cell viability at the end of twenty-four-hour period, seventy-two- hour MTT tests indicated glucose concentration-dependent decrease in metabolic activity. In glucose-only groups, a negative correlation was calculated between the MTT and neopterin/per cell. However a positive correlation was obtained between MTT/per cell and neopterin/per cell. Regardless of the presence of insulin, a negative correlation is evident between tryptophan and kynurenine. But we could not find any relationship between neopterin and kynurenine/tryptophan ratio. Decreases in neopterin per cell, tryptophan and kynurenine were tightly associated with genotoxicity. These findings suggested that the human U937 monocytic cells were most vulnerable to high glucose- induced oxidative stress. This study also showed that glucotoxicity is in genotoxic nature. On the basis of these data, neopterin and kynurenine biosynthesis of U937 cells does not necessarily relate to IFN-gamma stimulation, however insulin signalling-related system is in accordance with the high glucose utilization. The mechanisms of glucotoxicity related tryptophan depletion and cytokine release by U937 cells need to be clarified with further studies. Acknowledgement: This study was supported by Gazi University, Scientific Research Projects Division, 02/2011-32.

Keywords: U937 monocytic cells, glucotoxicity, genotoxicity, neopterin, tryptophan degredation

P-022 *ABSTRACT REF.: 083*

WHOLE GENOME SEQUENCING OF 16 TURKISH GENOMES REVEALS FUNCTIONAL PRIVATE ALLELES AND IMPACT OF GENETIC INTERACTIONS WITH EUROPE, ASIA AND AFRICA

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Turkey is a crossroads of major population movements throughout history and has been a hotspot of cultural interactions. Several studies have investigated the complex population history of Turkey through a limited set of genetic markers. However, to date, there have been no studies to assess the genetic variation at the whole genome level and without ascertainment bias. In this study, we present, for the first time, high coverage (32X to 48X) whole genome re-sequencing data from 16 individuals sampled from diverse geographical regions in Turkey, leading to the identification of 651,936 novel SNVs, 542,508 novel indels, and a non-redundant total of 10,731 deletion polymorphisms. Our results show that genetic variation within Turkey clusters with European populations, while containing signatures of admixture

from African and East Asian populations, consistent with influence of potential North African interactions and Altaic admixture. A number of variants associated with skin color and total cholesterol levels show frequency differentiation between the Turkish populations and European populations. Variations in the Human Leukocyte Antigen (HLA) loci between different regions of Turkey are also detected. Furthermore, we have analyzed the the 17q21.31 inversion polymorphism region(MAPT locus) and find increased allele frequency of 31.25% for H1/H2 inversion polymorphism when compared to European populations which shows about 25% of allele frequency. Our data will help develop population-specific experimental designs for studies investigating disease associations and demographic history of Turkey.

Keywords: whole genome sequencing, SNVs, indel, HLA

P-023 *ABSTRACT REF.: 094*

IS THERE ANY DIFFERENCE BETWEEN FLUOROMETRIC AND NEPHELOMETRIC ASSAYS FOR DETECTION OF C-REACTIVE PROTEIN LEVELS?

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C-reactive protein (CRP), has been extensively studied and found to provide a stable plasma biomarker for low-grade systemic inflammation, and its levels are upregulated in viral, bacterial and fungal infections, as well as in non-inflammatory conditions. In this study, difference between fluorometric and nephelometric assays detecting CRP level was investigated.

The patient specimens were analyzed with iCHROMA (i-CHROMA™, BODITECH Med inc., Korea) and IMAGE 800 (IMAGE 800®Beckman Coulter Inc., USA) analyzers. All systems were calibrated and quality control materials were analyzed according to the manufacturer's instructions. The results of the control materials were within the respective manufacturer's specified limits. The comparison studies were designed using CLSI EP9-A2 as a guideline.

Ninty six randomize serum samples were enrolled in this study. The IMAGE 800 and iChroma assays showed linear calibration graphs with correlation coefficient of r>=0,98 patient sera. Bland-Altman difference plot revealed good correlation. Seven data points were found outside the 95% limits of agreement. Here the mean difference was -6,9 percentage points with 95% confidence interval -20,7 to 6,7. Thus CRP levels of iChroma assays tended to give lower results. But, the limits of agreement (-1,96 and 1,96) were found to be confidential.

Several possible reasons could be suggested to explain why the seven data leaned out according to Bland-Altman. Firstly, the assays could be affected by manual process of sample preparing such as serum transfer on test cart. Secondly, light scatter/fluorescence of the CRP concentration in sera could not be reflected enough by iChroma. Thirdly, the study numbers of clinical samples which based our conclusion were relatively low, and study was conducted with randomized sample that patients' statues were unclear. Finally, all discordant results were positive in two methods and no patient would be adversely affected.

Keywords: C reactive protein, inflamation, diagnosis

P-024 *ABSTRACT REF.: 095*

COMPARISON OF TWO METHODS FOR PROCALCITONIN ANALYSIS

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Procalcitonin (PCT) is a 116 amino acid peptide that has an approximate molecular weight of 14.5 kDa and belongs to the calcitonin superfamily of peptides. In systemic bacterial and fungal infections, plasma concentrations are raised, whereas concentrations remain fairly low in infections of viral or nonspecific inflamations. In this study, randomized patient sera were used to evaluate simultaneously an automated PCT assay and a new semi-automated PCT assay with respect to their correlation.

A total number of 102 serum samples were analyzed according to the manufacturer's recommendations on both assays within 3 hours of receiving the specimens between November 2013 and February 2014. The patient specimens were analyzed with iCHROMA (i-CHROMA™, BODITECH Med inc., Korea) and mini-VIDAS (VIDAS®Biomerieux, France) analyzers. The mean serum PCT concentrations from the mini-VIDAS and i-CHROMA assays were 1.91 ±5.48 ng/ml (95% CI, 0,83-2.98) and 2.18 ± 5.88 ng/ml (95% CI, 1.03-3.34) respectively. The variance values were σ=30.01 and σ=34.59 for the mini-VIDAS and i-CHROMA assays, respectively. The concordance correlation coefficient value was 0.9451 (95% CI, 0.9206-0.9622). There was a highly significant correlation between the mini-VIDAS and i-CHROMA assays: r = 0.949 and 95% CI, 0.9247-0.9650 ($p < 0.0001$). The simple linear regression findings show the best-fit data points with exclusion of the highest PCT value (R2 = 0.945).

Etiology of sepsis in most cases is associated with bacterial or fungal infection; as such, culture and drug sensitivity testing often is considered the gold standard for diagnosis of infection. But culture reports cannot be available before 48-72 hours. This long lag period for culture results may cause delayed antibiotic therapy and increased morbidity/mortality for patients. Recent studies have demonstrated the potential of PCT as a parameter to guide antibiotic therapy in bacteremia. In this study, both mini-VIDAS and i-CHROMA were found to be ideal instruments for assessment of PCT.

Keywords: Procalcitonin, inflammation, bacteriemia

P-025 *ABSTRACT REF.: 092*

THE EFFECT OF H. PYLORI ON BAFF EXPRESSION FROM GASTRIC EPITHELIAL CELLS

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Helicobacter pylori (H.pylori) is a gram-negative and microaerophilic bacterium which is localized in the stomach. H.pylori is identified as one of the main risk factors for gastric cancer development. Around 80% of population in developing countries are infected with H.pylori and a subset of patients develop gastric complications such as chronic gastritis, mucosa associated lymphoid tissue (MALT) lymphoma etc. It is previously reported that B cell activating factor (BAFF) expression is upregulated in gastric epithelial cells by H.pylori colonization. To have a detailed understanding on H. pylori induced gastric immunopathology, firstly

we focused on the effect of H.pylori on BAFF expression from gastric epithelial cells. In order to investigate the role of H.pylori cytotoxin-associated gene A (CagA), one of the main virulance factors of H.pylori, on BAFF expression we treated two gastric epithelial cell lines [KATO-III, AGS] with sonicates of wild type H.pylori strain [G27] and its CagA mutant strain [ΔCagA] in time course experiments. Our data suggest that BAFF is upregulated in H.pylori-treated KATO-III and AGS cells after 24h or 48h, respectively. Also, conditioned media from H.pylori treated gastric epithelial cells were examined for their effect on cytokine secretion of Jurkat (T) cell line. Jurkat cells incubated with H.pylori-treated KATO-III conditioned medium secrete high levels of IL-17. Further research has to be performed to investigate the role of BAFF on this effect. Our initial data suggest that Interleukin 17 (IL-17) expression of Jurkat cells was significantly upregulated in supernatant of wild type H.pylori treated KATO-III cells. In conclusion, H.pylori sonicate treated KATO-III and AGS cell lines express BAFF and KATO-III cells' supernatants lead to IL-17 secretion from Jurkat cells.

Keywords: H.pylori, BAFF, gastric epithelial cell, T cell

IMMUNODEFICIENCIES

P-026 *ABSTRACT REF.: 064*

SCID PATIENTS WITH RAG DEFICIENCY FOLLOWING HSCT

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Severe combined immunodeficiencies(SCIDs) are heterogeneous group of inherited defects characterized by severe abnormalities in development and function of immune system. Patients present in the first months of life with severe, recurrent, and opportunistic infections, and without definitive treatment the condition is fatal. Some patients with severe combined immunodeficiencies (SCID) lacks both T and B cells while natural killer (NK) cells are normal. In these T-B-NK+SCID patients, defects have been identified in genes which are essential for V(D)J recombination. The majority of V(D)J recombination defects are caused by mutations in (recombination activating gene) RAG 1 or 2 and in DCLRE1C/ ARTEMIS genes.

Here we report a case of a RAG1 deficiency. A 4 months old boy was referred to our center with moniliasis, fever and diarrhea. On physical examination, he had severe moniliasis and growth retardation. He was the first child of parents with first degree consanguinity. Laboratory analyses; Hb:10.8 gr/dL,WBC:7800/ mm3,Plt:548000 /mm3,Absolute neutrophile count:4000/ mm3,Absolute lymphocyte count:900/mm3, IgG:3mg/dL, IgE:0.1 kU/L, IgM:4mg/dL, IgA:2mg/dL. Lymphocyte subtypes; CD 3: 1%, CD 19: 1%, CD20: 1%, CD16-56: 73%. Antibiotic prophylaxis and IVIG was started. His and parents´ DNA samples were sent to the University of Leipzig Immunodiagnostics lab. for molecular analaysis and reported as RAG1 mutation. His mother's HLA full-matched and after then HSCT was performed without conditioning regimen (bone marrow, TNC: 9,2x108/kg, CD34 13,6 x106/ kg). Before first admission to us, he was vaccinated with BCG and after HSCT, he developed left axillary lymphadenitis. Anti-tuberculosis drugs (INH, RIF) was started. On the follow-up he developed purpuras on the lower extremites. These purpura fulminans like lessions were assumed to be related with anti-tbc drugs and they were stopped. Low Molecular Weight Heparin was started. His liver function tests elevated with no associated viral panel and gastrointestinal GvHD was suspected. After immunosuppressive treatment his all function tests were within normal limits and we could start anti-tbc treatment (only INH). He has a complete chimerism after 3 months of HSCT.The curative treatment of patients with SCID is HSCT. HSCT survival depends on different factors such as early diagnosis, immunologic and clinical phenotype, pre and post HSCT supportive care, donor HLA typing and conditioning regimens. Our patient´s treatment was started at the 4 months of life with an HLA fullmatch HSCT without conditioning regimen. These factors importantly affects HSCT survival but he had been BCG vaccinated that could directly affects the morbidity.

Keywords: SCID, RAG, HSCT

P-027 *ABSTRACT REF.: 015*

BEHCET'S DISEASE IN A PATIENT WITH COMMON VARIABLE IMMUNODEFICIENCY: A CASE REPORT

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Common variable immunodeficiency (CVID) is one of the most common antibody deficiencies, is linked by a lack of immunoglobulin production, primary antibody failure and increased susceptibility to infection.. However, about 20% of patients with CVID develop an autoimmune complication. Behçet's disease (BD) is a rare vasculitis diagnosed by the presence of recurrent oral ulcers and two of the following: genital ulcers, typical eye lesions, and positive pathergy test. The patient was a 32 year-old female who admitted to the hematology department with fatique. General physical examination was unremarkable except for aphthous lesions of the oral mucosa. In medical history of the patient, she had genital area lesions periodically. Colonoscopy showed aphthous lesions in all colonic segments. Aphthous lesions were seen in the patient's oropharynx by esophago-gastro-duodenoscopy and she had BD diagnosis based on this findings. Screening tests of serum immunoglobulins showed decreased concentrations of four types of immunoglobulins: Total IgG 3.65, IgA 0.42, IgM 0.25 g/L. Serum protein electrophoresis showed hipogammaglobuline with gammaglobuline fraction in 4.8% and albumin/globuline ratio 1.44. In lymphocyte immunophenotype examination, CD3+ T cell ratio was 91.4%, CD4+/CD8+ 0.31, CD19+ 5% and CD16+56+ 3.2%. So, we detected a reduction in both the number and function of antibodies. The diagnosis was CVID. The patient received IVIG, colchicine. We present an uncommon case where a patient suffered from BD and CVID, together. This case is specific because of the two illnesses associated and only one case of an

association of BD and CVID reported thus far.

Keywords: Common variable immunodeficiency, Behcet's disease, autoimmunity

P-028 *ABSTRACT REF.: 121*

FRUCTOSE HAS THE ABILITY TO INDUCE GLUCOCORTICOID DYSREGULATION IN LIVER AND ADIPOSE TISSUE OF DIET-INDUCED METABOLIC SYNDROME RATS

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Metabolic syndrome (MetS) is a cluster of conditions—increased blood pressure, high blood sugar level and cholesterol levelsand an excess body fat—that occur together, increasing the risk of heart disease, stroke and diabetes. Recent studies prove that high level fructose consumption and accumulating glucocorticoid hormones play a significant role in the etiology of the diet-induced MetS. 11βHSD-1 is one of the tissue specific regulators of cortisol and glucocorticoid. 11βHSD-1 driven cortisone reactivation regulates adipose PAI-1 synthesis and secretion. The over expression of both in adipose tissue play a major role in the pathogenesis of MetS. In this study, we aimed to investigate and compare first the metabolic outcomes and hepatic (LT) omental adipose tissue (OAT) mRNA levels of 11βHSD-1 and PAI-1 genes upon fructose feeding under well defined conditions. Group I rats (n=10) were fed ad-libitum normal chow food and tap water and group II rats (n=10) fed with ad-libitum normal chow food and tap water containing 20% fructose for 15 weeks. In both group, the antropometric and biochemical parameters were analyzed at the beginning and at the end of the study. Further the dissection, 11βHSD-1 and PAI-1 mRNA levels were measured in OAT and LT by QRT-PCR. Our result showed that glucose, triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol, VLDL, ALT, AST, CRP, cortisol serum levels and HOMA-IR were significantly higher in group I compared to group II ($p<0.05$). Moreover, we determined a significant increase in OAT and LT 11βHSD-1 and PAI-1 mRNA levels in group I compared to group II ($p<0.05$) and a positive correlation between 11βHSD-1and PAI-1 mRNA levels in OAT ($r=0.684$, $p=0.029$). The skin thickness and waist circumference were both positively correlated with cortisol levels in group I starting from the seventh week. Furthermore, the PAI-1 mRNA levels were found to be positively correlated with cortisol levels in LT of group I ($r = 0.595$, $p = 0.006$). We conclude that, high fructose consumption for 15 weeks induces metabolic syndrome in rats and it also increases cortisol levels and mRNA levels of 11βHSD-1 in OAT which in turn might trigger an increase in PAI-1 expression in OAT. Finally, the interplay between fructose mechanism, the glucocorticoid action and PAI-1 might support the development of MetS.

Keywords: Fructose, Metabolic Syndrome, Liver tissue, Omental Adipose tissue, 11BetaHSD-1, PAI-1, Cortisol

P-029 *ABSTRACT REF.: 124*

EVALUATION OF THE IMMUNE SYSTEM IN AUTISM SPECTRUM DISORDERS (2 - 5 YEARS)

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Autism Spectrum Disorders (ASD), typically starting in the early years of life characterized by impairment in social interactions, communication deficits, and restricted repetitive interests and behaviors. ASD etiology remains unclear. Immune system is a community of cells which protect body from pathogens and malign cells. Increasing numbers of evidence pointed the relationship between ASD and immune system. In this study, expression of surface molecules, intracellular cytokine secretion and NK cell cytotoxicity were investigated. Expression of CD45, CD4 (T helper cells), CD8 (T cytotoxic cells), CD19 (B cells), CD16+56 (NK cells), CD3+HLA-DR+ (activated T cells),intracellular cytokine (IL-4, IL-10, IFN-γ) contents and natural killer cell cytotoxicity (NKCC) were measured in 29 ASD children and 13 typically-developing age-matched children. The severity of ASD was assessed with Childhood Autism Rating Scale. Compared to control group, CD45 ($p:0.002$), CD3 ($p<0.001$), CD4 ($p:0.022$), CD8 ($p<0.001$), IFN-γ+CD4+ T cell ($p<0.008$), NKCC ($p<0.001$) were significantly lower, IL-4+CD4+ T cells ($p<0.001$) were significantly higher in ASD. Correlated with severity of autism; there were higher and negative correlation with CD45, CD3, CD4 and CD8, lower and negative correlation with CD19, CD3+HLA-DR+ T cells, higher and positive correlation with IL-4+CD4+T cells, moderate and negative correlation with IFN-γ+CD4+ T cells, higher and negative correlation with NKCC. According to the immunologic parameters, there was no difference obtained between regressed and non-regressed ASD. Although pointing at additional evidence to immune system differences in ASD, our study was not able to determine whether these differences are factors playing role in the etiology of autism or develop secondary to chronic stress.

Keywords: Autism, immunprofile, flow cytometry

P-030 *[ABSTRACT REF.: 065*

TUBERCULOSIS AFTER BCG VACCINATION IN INFANTS WITH PRIMARY IMMUNODEFICIENCY

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BACKROUND
Bacillus Calmette-Guerin (BCG) is a attenuated live vaccine against tuberculosis, prepared from a strain of the bovine tuberculosis bacillus, Mycobacterium bovis. If BCG is accidentally given to an immunocompromised patient, it can cause disseminated or life-threatening infection. In this study we aimed to described the children with primary immunodeficiency diseases (PID) who were developed tuberculosis (TB) following BCG immunization.

METHOD
TB cases after BCG vaccination in PID infants are described retrospectively.

RESULTS
Between 2008-2011, 509 TB patients were treated in Ankara

Yenimahalle TB Dispensary. 17(3.3%) of them were children at 0-4 age group, and 5 (29.4%) of the infant cases were PID cases who diagnosed following BCG immunization. 3 infant had SCID, one had B and T cell immunodeficiency and the other one had IL-12 beta receptor deficiency. 3 of them were female and two of them were male. As we described the PID, all cases were born in term. There weren't any history of abnormalities at their perinatal periods and symptoms at postnatal periods before immunization by BCG. They were first or second child of their parents. Parents were not relatives. There was no history of TB in their families. Contact tracing was done to all their close relatives; no active TB case was found. Symptom onsets was between 1week to 6 months after BCG immunization. The symptoms changed as oral aphtha, fever, persistant wound, cervical/ axillary swelling to recurrent pulmonary or gastrointestinal infections. The cases diagnosed as TB, 3 weeks to 8 months after the onset of symptoms. Two cases developed miliary TB, two of them were TB lymphadenitis and one was soft tissue TB at vaccination area. Anti-TB treatment was started to all cases with first line drugs except pyrazinamid. Two of them had side-effects during anti-TB treatment. 4 cases were treated successfully, but one case with miliary TB died during the treatment.

CONCLUSION

It must be considered that TB after BCG vaccination may developed in infants with undiagnosed PID. Therefore, onset of regional lymphadenitis or wounds after BCG vaccination must be followed up carefully and PID kept in mind of physicians.

Keywords: Tuberculosis, BCG vaccination, Primary Immunodeficiency Disease

P-031 *ABSTRACT REF.: 005*

CUTANEOUS LEUKOCYTOCLASTIC VASCULITIS DUE TO SALMONELLA ENTERITIDIS IN A CHILD WITH INTERLEUKIN-12 RECEPTOR BETA-1 DEFICIENCY

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Mendelian susceptibility to mycobacterial disease(MSMD) is a rare immunodeficiency that is characterized by predisposition to infections caused by weakly virulent mycobacteria and Salmonella strains in otherwise healthy individuals.Here we report a case of an IL-12Rβ1 deficiency with cutaneous leukocytoclastic vasculitis due to Salmonella enteritidis.A four year old girl that had been diagnosed serologically with recurrent Salmonella infections,associated with lymphadenopathy and skin eruption was admitted as having Henoch-Schönlein purpura.She had been vaccinated with BCG and developed left axillary lymphadenitis which spontaneously drained and had recurrent oral monilia plaque. Edema and purpuric eruptions were present on the upper and lower extremities and the abdomen.Multiple mobile, painful,enlarged submandibular lymph nodes of about 2x2 cm in diameter were palpable.Skin biopsy showed a dense inflammatory site with eosinophils, neutrophils and fibrin in the upper dermis and dermal vessel wall,compatible with leukocytoclastic vasculitis.Serological studies to assess diagnostic markers for vasculitis and infectious agents were all negative.Immune work-up were unremarkable other than hypergammaglobulinemia. Salmonella enteritidis was identified in blood culture.She responded dramatically to ceftriaxone treatment within a few days and lesions cleared completely.Extended immunological and molecular genetic examination of the patient was carried out for IL-12/IFN-γ pathway defects.On the FACS analysis of Tcells for cell surface expression of the cytokine receptor chains, she did not express any IL-12Rβ1. Clinicians should be aware of possible infectious causes of vasculitis and children presenting with unusual recurrent infections caused by non-typhoidal Salmonella, BCG or nontuberculous mycobacteria, should be investigated for IFN-γ/IL-12 pathway defects.

Keywords: Leukocytoclastic Vasculitis, Salmonella Enteritidis, Interleukin-12 Receptor Beta-1 Deficiency

P-032 *ABSTRACT REF.: 030*

TWO NOVEL MUTATIONS OF BTK GENE IN TURKISH PATIENTS DIAGNOSED WITH X-LINKED AGAMMAGLOBULINEMIA

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X-linked Agammaglobulinemia (XLA) is a primary antibody deficiency characterized by severe reduction of immunoglobulins and mature B cells. Recurrent bacterial infections are often observed among patients. Mutation in the BTK gene, are detected in 85% of the patients in Western country, however the frequency in Turkey remains unknown. Here we assessed the BTK mutations in Turkish patients, which are diagnosed clinically as XLA,by DNA PCR and direct sequencing.Nineteen male patients with low level of B-cells were enrolled in this study. Total B-cell numbers and BTK protein was detected by flow cytometry. DNA PCR was performed to amplifythe BTK coding regions followed bydirect sequencing. Nine of 19 (47.36%) patients showed BTK mutations. Four of these mutations were deletions (c.441delT, c.713delG, c.1614delT, c.1464_1465delGA), one of them was an insertion (c.1519insT) andfour were single nucleotide mutation (c.491G>A, c.763C>T, c.1731A>C, c.1780G>A,). Seven mutations were previously identified whereas 2 mutations (c.441delT and c.1519insT) mutations were novel mutations. All detected mutations created a premature stop codon or a nonfunctional protein that causes a block in the B-cell development. In tenpatients, which no BTK mutation was detected, these patients might show an autosomal recessive formof agammaglobulinemia..

This study shows that DNA PCR followed by direct sequencing is an efficient and robust method for BTK gene mutations. Moreoverthis study also suggesting that BTK mutation is less common in Turkish patients compared to Western country. BTK gene mutation analyses are still on going in our department.

Keywords: PAD,XLA, BTK

P-033 *ABSTRACT REF.: 017*

EVOLUTION OF AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME BY FLOW CYTOMETRY

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The discovery of an unusual T-cell subset characterized by the expression of the α/β T-cell receptor without expression of either CD4 or CD8 [α/β-double-negative T cells (α/β-DNTCs)] provided critical insights in the evaluation of a lymphoproliferative disorder known as autoimmune lymphoproliferative syndrome (ALPS). The incidence and prevalence of ALPS are unknown. According to NIH data; ALPS affect less than 200,000 people in the US population. However, in Turkey the incidence of primary immunodeficiency disease including ALPS is thought to be much higher than US and Europe. ALPS is a disorder of defective Fas-mediated lymphocyte apoptosis, manifested by accumulation of α/β-DNTCs and other lymphocyte subsets, leading to autoimmunity and an increased risk of lymphoma. This is in contrast to the minor α/β-DNTC compartment in healthy individuals that contains multiple, immunophenotypically distinct subpopulations. In this study patients with a clinical diagnosis of ALPS were analyzed by Flow Cytometry. Peripheral blood samples obtained from patients, and α/β-DNTCs were evaluated by using anti-CD3, anti-CD4, anti-CD8 and anti-TCRαβ monoclonal antibodies and compared with healthy individuals. For a diagnosis of ALPS, a minimum 2.5% of T lymphocytes should be TCRαβ + DNTCs, in the setting of normal or elevated lymphocyte counts. Between 2009 and 2014, seven male (from 1 to 13 years) and five female (from 5 month to 11 years) patients were included into the study. Among the 12 patients, 2 females had α/β-DNTC percentage above 2.5 (11.14% and 29.00%) and in 2 patients (2 males) at borderline level (2.48% and 2.63%). In patients with chronic lymphoproliferation and autoimmunity analysis of double negative T cell by Flow Cytometry is a prompt and critical assay to investigate ALPS.

Keywords: autoimmune lymphoproliferative syndrome (ALPS), Flow Cytometry, α/β-double negative T cells

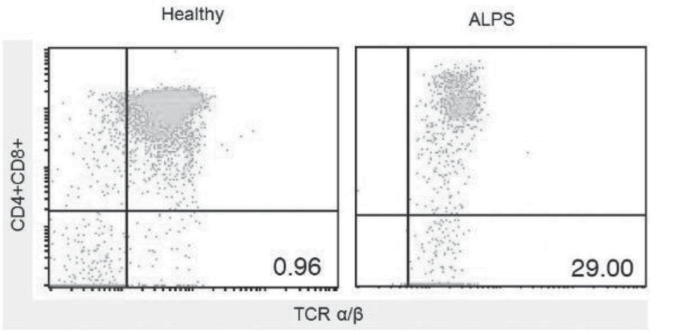

Fig 1. A flow cytometry output of a healthy and ALPS (SB 4.5 months, F) cases.

P-034 *ABSTRACT REF.: 037*

EVALUATION OF HYPER IMMUNOGLOBULIN M SYNDROME BY FLOW CYTOMETRY

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The hyper IgM (HIM) syndrome is an inherited immune deficiency disorder resulting from defects in the class switched recombination or somatic hypermutation process and is characterized by low serum concentrations of IgG and IgA and normal or elevated serum concentrations of IgM. X-linked HIM syndrome is caused by defects in the CD40L gene, while autosomal recessive HIM syndrome is caused by defects in the CD40. It is possible to diagnose patients definitely with genetic defects; but, flow cytometric assay can facilitate an accurate and timely diagnosis. In this study we analyzed CD40/CD40L expression by Flow Cytometry.

Peripheral blood samples obtained from patients and CD40+, CD19+CD40+ cells were determined using anti-19, anti-CD45 and anti-CD40 monoclonal antibody by Flow Cytometry according to whole blood lysing protocol. For diagnosis of X-linked HIM syndrome, isolated peripheral blood mononuclear cells were cultured 4 hours with PMA and Ionomycin, CD3+CD4+CD8-CD40L+ cells were stained using anti-CD3, anti-CD4, anti-CD8, anti-CD69 and anti-CD40L monoclonal antibody and analyzed by Flow Cytometry. All experiments studied with healthy donors' samples. For CD40 analysis twelve male (range: 1 to 20 years) and seven female (range: 3 months - 27 years) and for CD40L analysis eight male (range: 1-20 years) were included. Low CD40 expression in one female (0.09 %), and low CD40L expression on activated CD3+CD4+CD8- in 3 subjects (%0.03, %1 and %4 respectively) were observed.

The accurate diagnosis of HIM syndrome by Flow Cytometry is a quick and critical assay. Patients with recurrent infections accompanied with altered serum Ig concentrations should be investigated promptly to determine HIM syndrome.

Keywords: Hiper Immunoglobulin M Syndrome, Flow Cytometry, CD40 Ligand, CD40

INFECTION TO IMMUNITY AND VACCINATION: NEW INSIGHTS & DEVELOPMENT

P-035 *ABSTRACT REF.: 088*

INVESTIGATION OF PROTECTIVITY OF LIPOSOMAL HELICOBACTER PYLORI VACCINE CONTAINING NUCLEIC ACID TLR-LIGANDS

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Helicobacter pylori is a gram -ve bacteria, which is responsible for gastritis, peptic ulceration, and gastric cancer (70% of all gastric cancers). It infects 50% of the global population, 10% develop peptic ulcers, 1% gastric malignancies. Although the host elevates H. pylori specific IgG and IgA, eradication is rarely observed and might even impair host inflammatory responses and facilitate chronic bacterial colonization. There are no approved vaccines for H. pylori infection, furthermore, vaccines under investigation achieve partial control and does not provide any significant benefit from reoccurrence/recolonization of H. pylori in stomach. Therefore, new vaccines formulated with effective adjuvants are required. Nucleic acid based TLR ligands are initiating strong innate immune activation. However, their potential use in clinic due to in vivo degradation by nucleases or rapid clearance by serum protein adsorption is hampered. Encapsulation of these labile ligands within liposome not only increases their stability and protects them from pre-mature elimination but also enhances their immunostimulatory property. In this study, C57/BL6 mice were injected i.p. with free or liposomal TLR3 ligand (polyI:C) or TLR9 ligands (CpG ODN) together with H. pylori SS1 extract three times with one week intervals. IgG and IgA ELISA was performed using sera and feces samples collected at predetermined time points. The highest Th1 immune response (IgG2c/IgG1) was observed in mice immunized with (1555+SS1)SSCL. The highest IgA in sera was in mice immunized with (1555+SS1)SSCL & (pI:C+SS1)Neutral liposome. IgA was also higher in feces samples of mice immunized with (D35+SS)Anionic and (1555+SS1)SSCL. 15 days after last booster, animals were infected with H. pylori SS1 via oral gavage. After 15 days, they were sacrificed and their spleen and stomach were collected. The lowest H. pylori colony formation in stomach was observed in mice immunized with (1555+SS1)SSCL 15 days after infection. According to ELISPOT results, H. pylori specific IFNγ+ and IL17+ splenocytes were higher in mice immunized with (1555+SS1)SSCL, (pI:C+SS1)Neutral, and (SS1)Cationic formulations. In conclusion, the (1555+SS1)SSCL formulation was found to be an effective and promising vaccine candidate against the control of H. pylori infection/colonization.

Keywords: Liposome,TLR Ligands, Adjuvant, Vaccine, Helicobacter pylori

P-036 *ABSTRACT REF.: 072*

DELINEATION OF SYNERGISTIC IMMUNOMOSTIMULATORY EFFECTS OF LIPOSOMAL D- AND K-TYPE CPG ODNS ON HUMAN PBMC

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Liposomes are one of the best candidates for the encapsulation of labile bioactive agents due to their safety and high entrapment efficiency. Although both are strictly dependent on TLR9, two structurally distinct classes of CpG ODN are capable of activating human peripheral blood mononuclear cells (PBMC) by different signaling pathways. While K-type ODN trigger plasmacytoid dendritic cells (pDCs) to differentiate mature and produce primarily TNFα, D-type ODN lead to IRF-dependent IFNα secretion. When K-and D-type ODN are co-incubated in their free forms, K-ODN masks the D-ODN specific immune activation. Identifying proper delivery vehicles that provide both ODN types` to display their superior features upon stimulation is of great clinical importance. In this study, we investigated the synergistic effects of K- and D-ODN by encapsulating them within five different liposome types and aimed to detect their combinations during stimulation and identify synergistic activation on PBMCs.

PBMCs were isolated from 10 healthy individuals. Five different classes of liposomes were; i) neutral, ii) anionic, iii) cationic, iv) SSCL and v) stealth. Each liposome was loaded with D- and K-ODN by dehydration-rehydration method. Isolated PBMCs were stimulated with liposomes encapsulated with D- and/or K-ODN. Cytokines such as TNFα, IFNα, IP10 and IFNγ secretion were analyzed by ELISA, after 24 hours of stimulation.

Cytokine results revealed that D-ODN loaded in all five liposome types stimulated more IFNα than free D-ODN. Similarly, liposomal K-ODN triggered more TNFα than free K-ODN type. While incubation of free K and D- type ODN as expected, abrogated D-specific IFNα production from PBMC, simultaneous stimulation with neutral or anionic D-ODN loaded liposomes plus cationic liposomes loaded with K-ODN significantly increased rather than masking D-specific effect (i.e. more production of TNFα and IFNα specific for K and D). This pattern was observed in ~ 75 % of tested individuals. A second liposomal formulation composed of D-ODN loaded cationic liposome plus K-ODN loaded anionic or SSCL liposomes. Similarly, data revealed that, both TNFα and IFNα secretions were significantly increased in 25 % of tested individuals.

This study established that by selecting proper liposome type(s) we could establish a synergistic effect of D- and K-ODN mediated immunostimulatory activity in human PBMC. The retained superior features mediated by these CpG ODN classes, will critically contribute to design more effective immunotherapeutic strategies against health problems, ranging from cancer to allergy to vaccine adjuvant candidates.

Keywords: Liposome, CpG ODN, TLR9 Ligands, Adjuvant, Immunmodulation

P-037 *ABSTRACT REF.: 112*

ESAT-6 AND CFP-10 INDUCED CYTOKINE SECRETION OF MONOCYTES IN CHILDREN WITH PULMONARY TUBERCULOSIS

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Background. Mycobacterium tuberculosis (MTB) still represents one of the most important killers among the infectious pathogen. In children tuberculosis (TB) frequently disseminates and can be rapidly progressive early in life before immune competency is fully developed. While adult TB is commonly due to reactivation, pediatric TB is typically a primary disease and there are considerable differences in host immune responses in adults. Monocytes are antigen-presenting cells that play a crucial role in innate immunity to microbial infections and link innate with adaptive immunity. The aim of this study is to evaluate the monocyte phenotype together with their activation state and their cytokine secretion.

Material and Methods. The study group consists of pulmonary TB children (n=13, mean age=8 ± 5) and a healthy subjects (n=14, mean age=9 ± 5). Surface expression of CD16, CD14, HLADR, CD62L, CCR2 and CD163 in monocytes were evaluated. Whole blood was stimulated with ESAT-6 & CFP-10 and LPS for 16-24 h at 37°C. Intracellular TNF-alpha, IL-10, IL-12, IL-4 and IL-23 cytokine secretion of CD14+ monocytes were determined by flow cytometry. Mann-Whitney U test was used for non parametric values.

Results. Higher percentage of CD14++CD16+ and CD14+CD16++ monocyte subsets and also CCR2, CD62L and CD163 expression on circulating monocytes in children with PTB were obtained (p=0.005, p=0.0001, p=0.01, p=0.003 and p=0.002, respectively). Expression of CD14++CD16- and CD14++HLADR+ monocytes did not show any difference in both group. In PTB patients, compared to healthy subjects CD14+TNF-alpha+ and CD14+IL-10+ cells were significantly increased by ESAT-6 & CFP-10 stimulation (p=0.04 and p=0.03, respectively).

Conclusion. It is known that TB patients have alterations in their immune response, including monocyte phenotypic and functional changes. Our results showed that in childhood TB have an increased frequency of circulating intermediate and nonclassical monocytes compared to healthy controls. Increased level of CCR2 and CD62L might be affecting migration of monocytes in response to MTB inflamed tissues and secondary lymphoid tissues. Higher CD163 expression on monocytes might be a potential biomarker reflecting immune system in the resolution of inflammation and immune activation in TB. Increased levels of MTB antigen specific IL-10 and TNF-alpha secreting monocytes resembles both M1 and M2 type phenotype in childhood TB.

Keywords: Tuberculosis, monocyte, childhood tuberculosis, cytokines

P-038 *ABSTRACT REF.: 091*

MEAN PLATELET VOLUME AND NEUTROPHIL-TO-LYMPHOCYTE RATIO IN PATIENTS WITH CRIMEAN-CONGO HEMORRHAGIC FEVER

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OBJECTIVES: Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne zoonotic infection caused by Crimean Congo hemorrhagic fever virus (CCHFV). During viral hemorrhagic fevers, inflammatory processes are key elements of the immune response and related to the disease course. The aim of the present study is to investigate the association between blood neutrophil-to-lymphocyte ratio (NLR) and mean platelet volume (MPV) which are simple markers of subclinical inflammation and CCHF. We also investigated the relationship of these markers with coagulopathy parameters (activated partial thromboplastin time [aPTT], prothrombin time [PT], international normalized ratio [INR]).

METHODS: Thirty-one suspected CCHF patients, who submitted to Training and Research Hospital, Kayseri, Turkey between 2009 and 2013, were evaluated retrospectively. Among thirty-one patients, nineteen were laboratory confirmed CCHF patients diagnosed by RT-PCR or ELISA CCHFV-specific IgM positivity. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatinin phosphokinase (CK), INR, PT, aPTT, white blood cell counts (WBCs), and platelet counts of patient group were compared with twenty-five healthy individuals.

RESULTS: As shown in Table 1, MPV, AST, ALT, LDH, CK and coagulation parameters were significantly higher in patients with CCHF than the controls ($p<0.05$). WBCs, neutrophil, lymphocyte, platelet counts and NLR were significantly lower in patients with CCHF than the controls ($p<0.05$). We found no significant correlation between MPV, NLR and coagulation parameters.

CONCLUSIONS: Our study demonstrates that MPV and NLR may be beneficial markers in the diagnosis of CCHF. But these parameters should not be considered stand-alone tests for this use owing to non-specificity with other diseases.

Keywords: Crimean-Congo hemorrhagic fever, mean platelet volume, neutrophil-to-lymphocyte ratio

Table 1. Clinical and laboratory characteristics of CCHF patients and controls

	CCHF (n:19)	Controls (n:25)	p
Age (year)	45.8±10.6	41.7±13.6	NS
Gender (F/M)	6/13	13/12	NS
White blood cell, x10³/µL	2.5 (1.0-13.8)	7.38 (3.64-10.19)	< 0.001
Neutrophil, x10³/ µL	0.9 (0.4-6.9)	4.17 (2.36-8.22)	< 0.001
Lymphocyte, x10³/ µL	1.2±0.8	2.2±0.6	< 0.001
Platelet count, x10³/ µL	58.5±28.1	246.6±57.8	< 0.001
MPV, fL	9.9±1.4	8.8±0.7	0.003
NLR	1.4±1.1	2.2±0.9	0.011
PT (sn)	15.1±4.3	11.4±0.8	< 0.001
INR	1.2±0.3	0.9±0.1	0.002
aPTT (sn)	48.1±28.0	24.0±1.6	< 0.001
AST (IU/mL)	202.4±144.2	21.8±6.1	< 0.001
ALT (IU/mL)	102.8±58.5	24.6±13.2	< 0.001
LDH (IU/mL)	462.7±229.2	175.4±32.9	< 0.001
CK (IU/mL)	562.1±359.6	80.2±46.9	< 0.001

P-039 *ABSTRACT REF.: 126*

INVESTIGATION OF MRNA AND MIRNA EXPRESSION PROFILES THAT PLAY POTENTIAL ROLES IN THE DEVELOPMENT OF CHRONIC BRUCELLOSIS

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BACKGROUND: Brucellosis is a zoonosis, that is still endemic in developing countries. It can cause appreciable economic losses in the livestock industry because of abortions, decreased milk production, sterility, and veterinary care and treatment costs. Brucella bacteria are transmitted to humans by infected milk and dairy products of animals and caused disease. Therefore, Brucellosis also one of the most important public health problems. The most common clinical features of acute brucellosis are undulant fever, anorexia, weight loss, weakness, sweats, myalgias and arthralgias. Acute disease either recovers or becomes chronic form, similar to chronic fatigue syndrome, characterized with mild fever, sweating, weight loss and localized infections. Despite early diagnosis and treatment of patients, chronic infections are seen in 10-30% of patients. The purpose of this study, in the conversion of chronic infection that is effective is to determine the role of genetic factors.

METHODS: Approximately 45.000 genes and over 1,000 micro RNA (miRNA) were screened in peripheral blood mononuclear cells (PBMC) which were isolated from the peripheral blood of the patients with 8 acute and 8 chronic brucellosis with bone and joint involvement and 8 healthy controls, by using mRNA mikroarray and miRNA array. Quantitative real time polymerase chain reaction (qRT-PCR) was used to validate the expression of key molecules previously detected by microarray. RESULTS: In acute infection, expressions of 20 miRNAs and 753 mRNAs were significantly altered (more than two-fold compared to those of chronic infection). 19 miRNAs (miR-1972, -4485, -197-5p, -4497, -5739, -1973, -139-3p, -494, -211-3p, -513b, -584-5p, -652-5p, -3135-p, -4428, -4793-5p, -4656, -4433, -6069, -4257) were up-regulated, whereas only one (miR-1238-3p) was down-regulated ($p<0.05$).

CONCLUSIONS: Establishment of clinical usability of altered gene and miRNA expressions may provide alternative choices for following up and treatment of disease and determining the prognosis. This may also lead to development of new treatment strategies especially for chronic brucellosis.

Keywords: Brucellosis, peripheral blood mononuclear cells (PBMC), acute infection, chronic infection, gene expression, Micro array, miRNA

P-040 *ABSTRACT REF.: 021*

THE CELL AND MOLECULAR ASPECTS OF EFFICACIOUS PROTECTIVE IMMUNITY ON IMMUNOCOMPROMISED HOST AGAINST INTRACELLULAR INFECTION

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Francisella tularensis (F. tularensis) is a highly virulent, intracellular pathogen. An efficient immune response is dependent on T cell-mediated immune responses and IFNgamma production during first days after F. tularensis LVS (LVS) infection. Nevertheless, there is also an evidence that B cells, as well as antibodies, are necessary for mice to develop a resistance against primary and secondary infection by LVS.

Here we demonstrated that B cells directly interact with F. tularensis LVS microbes and, as a consequence of this interaction, B cells immediately produce antibody clones directed to F. tularensis proteins. We also utilized gamma-irradiated mice model for studies of the protective role of anti-F. tularensis antibodies in order to partially eliminate cellular responses and also address the responses in immunocompromised host. The passive transfer of immune sera from LVS immunized mice protected sublethally irradiated mice against the challenge with otherwise lethal LVS infection, led to the decrease of cytokine serum levels, increased the level of IFNgamma and, conversely, had a minimal effect on the levels of these cytokines in organ homogenates when compared to the nonimmunized counterparts.

In summary, we demonstrate that B cell-mediated effector responses together with parallel induction of T cell-mediated immunity both play an important role and this should be taken into the account as a powerful strategy in the design of new vaccines.

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Keywords: Immunity to bacterial infection, Francisella tularensis, B cells, gamma irradiation

P-041 *ABSTRACT REF.: 100*

MYCOBACTERIUM TUBERCULOSIS MANNOSE CAPPED LIPOARABINOMANNAN INDUCED MACROPHAGE POLARIZATION IN FIBROBLAST - MACROPHAGE COCULTURE MODEL

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Backround Mycobacterium tuberculosis is a highly significant human pathogen that latently infects billions of people and causes active contagious and chronic granulomatous disease in millions of patients worldwide. In the pathogenesis of tuberculosis, granulomas play a key role, and macrophages play an essantial role in protection to Mycobacterium tuberculosis. M.tuberculosis-macrophage interactions are key in the clearance of the bacteria. Especially, interactions between M. tuberculosis mannose-capped lipoarabinomannan (ManLAM) and macrophage receptors modulates macrophage functions. In these study, we aimed to

evaluate the effect of the fibroblast, the cells serve a restriction founction in the granuloma formation, to the macrophage polarization in a coculture model.

Method ManLAM were isolated from M. tuberculosis H37Rv standart strain. Firstly, H37Rv cells delipitated using methanol / chloroform, and then the lipitated cells extracted by refluxing in ethanol. After removing the proteins, the extract subjected to Triton-X phase seperation. The ManLAM monitored by SDS-polyacrylamide gel electrophoresis.

3T3 mouse fibroblast cell line and J744.1 mouse macrophage cell line were used in the coculture studies. The cells treated with ManLAM in three different conditions; alone, together and together with an 0.4 µm pore size nylon membrane filter. After 18 hour incubation, supernatant collected for cytokine analysis. After 24 hour incubation, cells prepared for cell surface receptor analysis with flow cytometry

Results Macrophages treated with ManLAM in the presence of fibroblast after a period of cell incubation produced IL-12 and nitric oxide, markes of the proinflammatory type-1 macrophages (M1). Also the MHC class II and CD86 expression of the macrophages treated with ManLAM in the presence of fibroblast were decreased if compared with the macrophages in the absence of fibroblast.

Conclusion The data demonstrated that, the fibroblasts had an effect in the macrophage M1 polarization in a contact depended manner.It is suggested that, fibroblasts had a very important and immunologically active role in the granuloma formation.

Keywords: Mycobacterium tuberculosis, ManLAM, fibroblast, macrophage, coculture

MICROBIOME IN HEALTH AND DISEASE

P-042 *ABSTRACT REF.: 079*

IMMUNOMODULATORY AND ANTI-INFLAMMATORY ACTIVITIES OF COMMENSAL BACTERIA-DERIVED MEMBRANE VESICLES

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Gram negative pathogenic bacteria constitutively secrete membrane vesicles that are enriched in TLR ligands (such as peptidoglycans, LPS and nucleic acids) and thus contribute to immune activation. In contrast, the immunomodulatory properties of MVs secreted from human commensal bacteria remain largely unknown. Given the importance of microbiota as regulators of immune homeostasis, we aimed to assess the immunomodulatory properties of extracellular vesicles secreted by 3 different human commensal lactobacilli isolates in comparison to E.coli derived outer membrane vesicles. In order to understand how MVs could contribute to Ag-specific immune responses, mice were immunized with an inactivated viral vaccine against the foot an mouth disease virus together with commensal or E.coli-derived MVs. Results showed that FMD-specific IgG2a responses were suppressed when the vaccine contained MVs derived from commensals but not from E.coli. Similarly, commensal MVs suppressed anti-OVA IgG2c antibody responses in OVA immunized mice and exacerbated tumor progression following challenge with EG.7 tumor cells, suggesting that commensal-derived MVs ameliorate Th-1 dominated inflammatory responses. To test the immunomodulatory activities of commensal-derived MVs in an antigen independent chronic inflammation model, their anti-inflammatory activities were tested in a CCl4-induced liver fibrosis model in C57BL/6 mice. Results showed that, MV treated groups had decreased αSMA expression in the liver both at the mRNA and at protein levels. ALT levels of these groups were also found to be significantly lower than the animals in the fibrotic group. Splenocytes of fibrotic mice produced 2.6-fold more TNFα than untreated controls, whereas MV treated fibrotic mice showed a significant reduction in TNFαresponse (only 1.5 fold increase with respect to the control group). These results indicate that human commensal bacteria-derived membrane vesicles can have powerful immunomodulatory effects and can have potential therapeutic applications as novel anti-inflammatory agents.

Keywords: membrane vesicles, commensal bacteria, immune regulation

P-043 *ABSTRACT REF.: 090*

EXOPOLYSACCHARIDES FROM COMMENSAL BACTERIA ALTER TLR MEDIATED TH1 IMMUNITY

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Exopolysaccharides (EPS), one of the primary metabolic products of bacteria, were shown to possess immunomodulatory and antitumoral activities. Membrane bound (EPS-B) and released (EPS-R) exopolysaccharides were extracted from Pediococcus. Immunomodulatory effects EPS-B and EPS-R were studied in both mouse splenocytes and human peripheral blood mononuclear cells (hPBMC) in vitro. Both types of EPS failed to induce any detectable Th1-dependent cytokine production by immune cells. Next, we co-incubated TLR3 ligand; pIC (double stranded RNA), TLR9 ligands; D (D35) or K (K23) type CpG ODNs with EPSs to test whether they act in antagonistic or synergistic manner with these ligands. Stimulation with pIC, D35 or K23, co-incubated either with bound or released forms of EPSs significantly suppressed IL-6, IL-12 cytokine productions in mouse splenocytes and IP-10, IFN-α release from hPBMC at least 3 fold as shown by ELISA. To discriminate which fraction of EPS contributes to observed immunosuppressive effect we ultracentrifuged EPSs and co-incubated pellet and supernatant fractions with the TLR ligands. IP-10 and IFN-α production from hPMBCs which were incubated with pIC/D35+pellet EPS were abolished ~80% compared with pIC and D35 alone responses. Supernatant of EPSs were also suppressive albeit at much higher levels. When incubated with TLR3 and TLR9 ligand supernatant EPS gave ~40-50% suppression. In vivo immunomodulatory action of EPS/CpG ODN combinations were tested against model antigen ovalbumin (OVA) in BALB/c mice. Mice immunized with EPS-R/EPS-B+D35+OVA and EPS-R/EPS-B+K23+OVA formulations showed Th-2 dominant anti-OVA immune responses as evidenced by higher IgG1 and lower IgG2a six months after booster i.p. injection. Taken together these data suggested that a commensal microbe-derived EPS could drive a Th-2 biased anti-OVA immune response when used together with an established Th-1 based adjuvant(s). The main components accounting for immunosuppressive effects of these EPS should be further investigated.

Keywords: exopolysaccharide, immunosuppression, adjuvant, TLR, commensal bacteria

P-044 *ABSTRACT REF.: 082*

DETERMINATON OF THE FREQUENCY OF ROTAVIRUS AND ADENOVIRUS IN CHILDREN WITH ACUTE GASTROENTERITIS AND MOLECULAR EPIDEMIOLOGY OF ROTAVIRUS

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Enteric viruses (especially Rotavirus, Norovirus and Adenovirus) are the most common causes of non-bacterial acute gastroenteritis. Aim of this study was to investigate the prevalence of the viral etiology of the gastroenteritis in children aged 0-6 years with acute gastroenteritis and determine predominant genotypes of Rotaviruses.

An epidemiological study on common diarrheal viruses was carried out in province of Afyonkarahisar, Turkey, during September 2012-November 2013. Fecal samples obtained from the 492 unvaccinated children with rotavirus vaccine and under 6 years of age who applied to the Pediatric Diseases Outpatient of Afyon Kocatepe University Hospital with the complaint of diarrhea were enrolled into the study. All the fecal samples were evaluated in order to determine presence of the bacterial agents by using bacteriological culture methods and finally no growth observed. Rotavirus and Adenovirus antigens were examined by immunochromatographic method (ICT) (VIKIA Rota-Adeno Casette Test, bio-Merieux, Marcy l'Etoile, France) in stool samples. Rotavirus positive Stool samples with ICT were subjected to reverse transcription-polymerase chain reaction based genotyping of the outer capsid antigens, VP7 and VP4, determining G and P type specificities, respectively.

Adenoviruses and Rotavirus were found to be positive in 3.3 %, and 20.3 % of 492 children with acute gastroenteritis respectively. Of the children with viral gastroenteritis, 3 % had a mixed adenovirus-rotavirus infection. An increase in the number of cases with rotavirus antigen-positivity was detected in winter and spring months. Of the Rotavirus episodes, 73.0 % were occured during the first two years of life. A total of six different combinations of G and P types were found that included those with combinations of G1, G2, G4, G9 and P[4], P[8] genotypes. The most common rotavirus genotypes were G9P[8] (48.7 %), followed by G9P[4] (17.5 %). Other strains were G1P[8] (16.2 %), G2P[8] (11.2 %), G1P[4] (3.7 %), G4P[8] (2.5 %). Sixty-six percent of our regional rotavirus genotypes were G9P[8] and G9P[4].

The frequency of the positivity of adenovirus was lower than rotavirus. Regarding high frequency rotavirus infection, continuous surveillance is needed in order to inform gastroenteritis prevention programs as well as to provide information about the occurrence of new rotavirus strains. This will assist policy makers in decision making on rotavirus vaccination.

Keywords: Acute gastroenteritis, rotavirus, adenovirus, genotyping

P-045 *ABSTRACT REF.: 085*

AN INVESTIGATION OF CARBAPENEMASE GENES IN CARBAPENEM RESISTANT KLEBSIELLA PNEUMONIAE STRAINS

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Carbapenem-hydrolysing beta-lactamases are the most powerful beta lactamases and they are able to hydrolyze almost all beta lactams. They are mostly of the KPC, VIM, IMP, NDM and OXA-48 types. Phenotypic and molecular-based techniques are able to define these carbapenemase producers. In this study; it was aimed to investigate carbapenemase gene regions responsible for production of carbapenemase in carbapenem-resistant Klebsiella pneumoniae isolate. The study was performed at Afyon Kocatepe University Hospital in Turkey between December 2013 and March 2014. Twenty-two carbapenem resistant K. pneumonia strains isolated from various clinical samples were investigated. All non-duplicate clinical isolates of K. pneumoniae isolated from inpatients that showed decreased susceptibility to any of carbapenem were collected during the study period. Identification of the bacteria and antibiotic susceptibility tests were performed by conventional methods and automated systems (Phoenix100, Becton Dickinson Co., Sparks, MD, USA). Conventional PCR were used to detection of the 23s rRNA and KPC, VIM, IMP, NDM and OXA-48 genes encoding carbapenemase.

K. pnemoniae strains were isolated from bloods (n=5), wounds (n=7), sputums (n=4), tracheal aspirates (n=1), and urine samples (n=5). All the isolates were resistant to ertapenem. Three isolates were sensitive to both the imipenem and meropenem, while the others were resistant or had decreased susceptibility at least each one. OXA-48 encoding genes were detected in all K. pneumoniae isolates. The co-existence of two or three different carbapenemases genes was observed. The co-existence of blaOXA-48+blaVIM-type genes were detected in 6 strains and blaOXA-48+blaVIM-type blaIMP-type genes in 4 strains.

The worldwide spread of Enterobacteriaceae expressing carbapenemases represents a major important threat of global health concern, because these carbapenemase producers are resistant to most of the antibiotics. Detection of carbapenemase producer strains are the two main approaches for prevention of their spread, although with variable efficiencies. The results of this study indicate that, the occurrence of carbapenemases in carbapenem resistant K. pneumoniae isolates are significantly higher and blaOXA-48 was the most common genes encoding carbapenemase in our hospital.

Keywords: Carbapenem resistance, K. pneumoniae, 23s rRNA, carbapenemases

P-046 *ABSTRACT REF.: 027*

INVESTIGATION OF PANTON-VALENTINE LEUKOCIDIN IN COMMUNITY-ACQUIRED AND HOSPITAL-ACQUIRED STAPHYLOCOCCUS AUREUS IN SOUTHEAST OF TURKEY

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Staphylococcus aureus causes serious hospital and community-acquired infections. Especially skin and soft-tissue infections are sometimes related with strains harboring Panton-Valentine leu-

kocidin (PVL). PVL belongs to a family of similar bi-component leukocidal toxins produced by staphylococci. It is a pore-forming toxin encoded by lukF-PV and lukS-PV.

A total of 70 S.aureus strains (38 (54%) MRSA, 32 (46%) MSSA) were isolated from the patients admitted to Dicle University Hospital. Identification of S.aureus and antibiotics-susceptibility testing were performed with PHOENIX 100. PVL genes and mec A genes were detected by PCR. Of 70 strains 36 strains (51%) were CA (Community acquired) and, 34 (49%) strains were HA (Hospital acquired). A total of 38 (54%) strains were positive for mecA. Among mecA (+) strains 32 (%84) were HA. Among mecA(-) strains 30 (94%) were CA. Of 70 study strains, 12 (17%) strains were PVL (+) (8CA, 4 HA). Among 36 CA strains 8 (22%) strains were PVL(+), among HA strains 4 (12%) were PVL (+). Among 12 PVL(+) strains, 4 strains were mecA (+). Among methicillin susceptible strains PVL positivity rate was 25% (8/32), among methicillin resistant strains. PVL positivity rate was 10.5 % (4/38). Of PVL (+) strains, 7 strains were obtained from wound, 4 skin abscess, 1 blood culture.

In conclusion identification of Panton-Valentine leukocidin positive Staphylococcus aureus and an appropriate therapy can reduce mortality. Further research is needed to specify the types of SCCmec and detect Panton-Valentine leukocidin positive Staphylococcus aureus infections in our region.

Keywords: PVL, PCR, S.aureus

NEUROIMMUNOLOGY

P-047 *ABSTRACT REF.: 011*

POSSIBLE ROLE OF UNCOUPLING PROTEIN 2 IN MULTIPLE SCLEROSIS

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INTRODUCTION AND AIM: Multiple sclerosis (MS) is a chronic disease which develops due to autoimmune mechanisms triggered by environmental factors in sensitive individuals, which may involve multiple regions of the central nervous system. The cause of MS is not clearly known, in spite of extensive investigations. Uncoupling protein 2 (UCP2) gene is present in the mitochondria, on the long arm of 11th chromosome (11q13). UCP2 is a mitochondrial carrier protein which provides the proton conductivity opposite the inner mitochondrial membrane, whose main function is the control of mitochondrial reactive oxygen species. Although not conclusively shown, its physiological role is thought to be maintenance of calcium homeostasis, regulation of neuronal activities and prevention of cellular damage. These factors are very important in the determination of outcome of nerve cells and brain damage in neurodegenerative diseases. Aim of this study was investigation of UCP gene expression levels and delineation of a possible relationship with MS development.

MATERIAL-METHODS: mRNAs were obtained from the peripheral blood samples of 95 patients with MS and 95 healthy individuals without any neurodegenerative diseases, and expression levels of UCP2 gene of the two groups were compared by RT-PCR. The multiple data obtained were analyzed by Mann – Whitney U test, after ratio of patient / healthy control results were calculated and GAPDH and Beta – Actin normalization.

FINDINGS AND DISCUSSION: The gene expression was found to be significantly decreased (p=0.0000024) in the patient group after the statistical analysis. UCP2 blocks the production of ATP, thus permitting output of the energy in nutrients only as heat. New evidence suggests that an increase in the UCP2 gene expression and activity is associated with stroke and neuronal survival after trauma. The expression of UCP2 gene was found to be significantly decreased in the patient group ($p < 0.05$) in the present study, and further analysis were planned for future following the completion of the study. UCP2 and the genes that they interact in the pathways are considered to be promising for the diagnosis and treatment of MS.

Keywords: Uncoupling Protein 2, Multiple Sclerosis, RT-PCR

P-048 *ABSTRACT REF.: 047*

FUNCTION OF NF-KB2 GENES IN NEURODEGENERATION

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INTRODUCTION AND AIM: Multiple Sclerosis (MS), which is among neurodegenerative disorders, is an inflammatory demyelinizating disorder which occurs as a result of entrance of activated myelin-specific T cells into the central nervous system (CNS) and is characterized by demyelinized plaque formation at the white matter. The expression levels of 2nd and 3rd variants of the nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (NF-kB2) gene in MS as a model of neurodegenerative disease were compared with the control group in the present study. NF-kB increases the expression of various genes which support survival of neurons such as IAP (inhibitor of apoptosis) Bcl-2 and calbindin, by interacting with multiple pathways. On the other hand, apoptosis pathway induced by TNF-α is entered by deacetylation of NF-kB. Prevention of NF-kB in the neurons accelerate the neurodegenerative process.

MATERIAL-METHODS: mRNA were obtained from the peripheral blood samples of 95 patients with MS and 95 healthy individuals without any neurodegenerative diseases, and expression levels of 2nd and 3rd variants of the NF-kB2 gene of the two groups were compared by RT-PCR. The multiple data obtained were analyzed by Mann – Whitney U test, after ratio of patient / healthy results were calculated and GAPDH and Beta – Actin normalization.

FINDINGS AND DISCUSSION: The expressions of NF-kB2*2 (p:0,0000431) and NF-kB2*3 (0,0000959) genes were found to be significantly decreased (p<0,05), after the statistical analysis. Further analysis were planned in the process following the completion of the study. NF-kB family and the genes that they interact in the pathways are considered to be promising for the diagnosis and treatment of MS.

Keywords: NF-kB2, RT-PCR, Neurodegeneration, Multiple Sclerosis

P-049 *ABSTRACT REF.: 108*

NATURAL KILLER CELLS: DO THEY HAVE ANY IMMUNE REGULATORY EFFECTS IN MULTIPLE SCLEROSIS?

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Multiple sclerosis (MS) is an inflammatory disease of central nervous system. Although the pathogenesis of the disease is not well known, there are studies that indicate the contribution of the immune system. Natural killer (NK) cells are shown to have immunoregulatory or immunsuppressive roles in the pathogenesis of autoimmune diseases, but the actual role of NK cells in MS pathology is not clearly revealed yet. Our study aimed to investigate the functions of CD16+CD56dim and CD16-CD56bright NK cell subsets in different subgroups of MS. For this aim, firstly, cell surface expression levels of CD3, CD4, CD8, CD19, CD16 and CD56 were detected in peripheral blood samples, and peripheral blood mononuclear cells were cultured for 24 hours in the presence and absence of hrIL-2, hrIL-4 and hr IL-12. After the cell culture, IFN-g, IL-10 and IL-22 contents of CD16-CD56bright and CD16+CD56dim NK cell subsets were measured by flow cytometry. NK cytotoxicity was measured using erythromyeloblastoid leukemia cell line K562. Compared to healthy subjects, higher IL-10 and IL-22 levels in CD16+CD56dim and CD16-CD56bright NK cell were detected in MS patients. On the other hand, significantly decreased IFN-g levels of CD16-CD56bright NK cell subset were measured in response to IL-12 stimulation in all patient groups compared with healthy controls. In addition, cytotoxic activities of NK cells were significantly decreased in all patient goups. Increased IL-10 and IL-22 secretion by NK cell subsets in MS patients revealed that they might have a suppressive effect such as inhibiting synthesis of pro-inflammatory cytokines. In addition, decreased cytotoxic activity of NK cells in patients as well as decreased IFN-gamma production in response to stimulation in all patient groups underline possible regulatory roles of NK cells in MS pathogenesis.

Keywords: Multiple Sclerosis, NK cells, Inflammation, Cytokines, Flow Cytometry

P-050 *ABSTRACT REF.: 052*

CIRCULATING FOLLICULAR HELPER T CELLS IN MYASTHENIA GRAVIS

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Myasthenia gravis (MG) is an autoimmune condition characterized by muscle weakness due to the production of autoantibodies, such as those against the acetylcholine receptor (AP) and muscle-specific tyrosine kinase (MP). T follicular helper (Tfh) cells are the specialized CD4+ T cell subset that induces the activation and differentiation of B cells into immunoglobulin (Ig) secreting cells. Tfh cells are found in germinal centers (GCs), express high levels of C-X-C chemokine receptor type 5 (CXCR5), programmed death-1 (PD-1), and inducible costimulatory molecule (ICOS). In particular after the identification of Tfh cells in the peripheral blood, we are interested in the role of Tfh cells in the pathogenesis of MG.

Tfh cells of patients with different autoantibody production, age of disease onset and immunosupressive (IS) treatment are investigated and compared with healthy controls (CON). Patients with early-onset (EOMG, younger than age 50) and late-onset (LOMG, older than age 50) are also included. In this study, expression of CD70, ICOS, PD1, IL21R, ICOSL and PD1L1 are evaluated on CD4+ T cell and CD19+ B cell. All patients and healthy control characteristics summarized in Table 1.

CD4+T cells was lower in MP (p= 0.002 and p=0.032, respectively) than in CON and AP and this finding held true in MG patients with corticosteroid treatments in compared with IS (-) and CON (p= 0.45 and p= 0.003, respectively). AP, MP and EOMG groups had lower CD4+CXCR5+ Tfh than CON (p= 0.013, p=0,002 and p=0.002, respectively). ICOS and CD70 expressions in Tfh were higher than CON. Also, ICOS expression on Tfh was higher in AP EOMG IS (-) group. CD19+CD27- (naive B cells) were significantly lower in AP, MP patients with IS treatment than CON. PD1, PD1L1, CD70, ICOSL expressions are increased in naive B and associated with IS treatment. PD1, PD1L1, ICOSL and IL21R expressions are increased in naive B of LOMG patients versus CON. ICOSL, PD1, CD70 expressions are decreased in CD19+CD27+ (memory B cells) of EOMG vs. LOMG and CON. PD1L1 expression is increased in CD19+CD27+ of LOMG vs. EOMG and CON but these differences are not seen in the AP group.

Based on the finding, immunosuppressive treatment effects the B cells also specially naive B cells and the lower levels of B cell express higher costimulatory molecules.

TUBITAK HAS SUPPORTED THIS STUDY.

Keywords: Follicular helper T cells, Myasthenia Gravis

Patients and healthy control characteristics

		AP	MP	CON
EOMG	IS (+)	9		
	IS(-)	11		
LOMG	IS(+)	11		
	IS(-)	5		
TOTAL		36	20	21

P-052 *ABSTRACT REF.: 137*

A KEY ELEMENT OF ENDOCANNABINOID SYSTEM PPARγ2 AND LEPTIN ARE ASSOCIATED IN TURKISH SCHIZOPHRENIA PATIENTS

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BACKGROUND: The endocannabinoid system (eCB) is deeply involved in body weight regulation, other than the obesity genes, therefore endocannabinoid genes may also have a role in the antipsychotic-induced weight gain in schizophrenia(SCH)patients.

AIM: To further investigate this hypothesis, we performed an association study with PPARγ2 gene codifying for a key element of the eCB system and obesity related genes, leptin, leptin receptor and MC4R, in 320 SCH patients and 437 controls.

METHODS: Biochemical analyses, the effects of LEP c.-2548G>A, LEPR c.668A>G, PPARγ2 c.-2-28078C>G, MC4R c.307G>A polymorphisms and the impact of those genes mRNA and serum levels on metabolic adversities in SCH patients and control groups were studied.

RESULTS: Significantly higher BMI and fasting blood sugar and significantly lower HDL levels were present in SCH patients compared to controls (p<0.001). PPARγ2 and LEP polymorphisms were significantly different between SCH and control groups, while a significant difference was present in PPARγ2 polymorphism between male SCH patients and male controls (p<0.005) and LEP polymorphism between male and female SCH patients (p<0.005). Interestingly, MC4R c.307G>A (G/A+G/G) carriers showed significant differences in BMI and LEP mRNA levels compared to wild type SCH patients (A/A). LEP, LEPR, PPARγ2 mRNA levels and leptin serum levels were also significantly higher in SCH patients compared to controls (p<0.001,respectively). Leptin serum and mRNA levels were positively correlated in PPARγ2 c.-2-28078C>G carriers (p<0.001) and LEP c.-2548G>A carriers have significantly higher PPARγ2 mRNA levels (p<0.001).

CONCLUSION: Our findings suggest a strong association between PPARγ2 and leptin genes in Turkish SCH patients which might indicate their potential role in the antipsychotic-induced weight gain, but further studies are needed in order to elucidate their involvement in the pathophysiology of SCH.

Keywords: leptin, schizophrenia, endocannabinoid system, pparγ2, antipsychotic drug

P-053 *ABSTRACT REF.: 026*

VITAMIN D RECEPTOR FOK1 GENE POLYMORPHISM AND RISK OF SUBACUTE SCLEROSING PANENCEPHALITIS

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Subacute sclerosing panencephalitis (SSPE) is subacute inflammatory and neurodegenerative encephalitis related to the measles virus and usually affecting children and young adults. Although the exact pathogenesis of SSPE remains to be determined, previous studies suggested a genetic contribution to the host susceptibility to SSPE. Vitamin D regulates cellular activity, also it has effects on: innate and adaptive immunity, antimicrobial, anti-inflammatory and immunomodulatory functions. Vitamin D deficiency has been showed to cause reduction of chemotaxis, phagocytosis and proinflammatory cytokine production. It is known that variations in VDR gene affect resistance or susceptibility to infections such as HIV-1, HBV, HTLV-1 and tuberculosis. The purpose of our study was to elucidate the role of polymorphisms in the Vitamin D receptor gene polymorphism in the development of SSPE. Using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, Vitamin D receptor gene polymorphism was studied in 52 patients with SSPE and 90 healthy controls. Analysis of the Fok-1 polymorphism of the VDR gene revealed that the frequency of the F allele (72.2%) was significantly higher in the control group, whereas the frequency of the f allele (39.4%) was significantly higher in the SSPE patients (p=0.048, odds ratio [OR]: 1.692). However, the distributions of the three genotypes (F/F, F/f, f/f) in patients with SSPE (38.5%, 44.2%, 17.3%) did not differ significantly from those in the normal controls (52.2%, 40.0%, 7.8%). In conclusion, these data suggest that the Vitamin D receptor polymorphism might be genetic risk factor for the SSPE disease.

Keywords: Subacute sclerosing panencephalitis, polymorphism, VDR.

P-054 *ABSTRACT REF.: 118*

FUNCTIONAL SUBSETS OF T CELLS ARE ALTERED IN SUBACUTE SCLEROSING PANENCEPHALITIS PATIENTS

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Subacute sclerosing panencephalitis (SSPE) is a persistant virus infection caused by measles virus (MV). In addition to viral factors, host factors seem to contribute to the development of SSPE. Altered immunoregulatory mechanisms can be responsible for the failure of immunosurveillance in children with SSPE. During measles infection, a switch from a Th1 to a long-lasting Th2 response has been demonstrated. The ratio of these cell subtypes as well as regulatory and suppressor cell functions are investigated in persistence of MV.

In this study, 52 children with SSPE were compared with age matched 57 children (CON). The mean age of SSPE patients was 10.0±4.8 years. In the control group, the mean age was 10.1±6.1 years. Peripheral blood mononuclear cells (PBMC) were isolated from the donors and surface marker expression for CD4+ Th1, Th2, CD8+ and Treg cell subsets have been evaluated among the groups phenotypically by flow cytometric analysis. Results were analyzed by using the non-parametric tests and mean±SD values are presented.

The frequencies of total CD3+ T cells in SSPE patients were lower in SSPE patients compared to controls (59.1±12.2% vs. 65.0±10.8%, p=0.043), whereas the frequencies of CD4+ and CD8+ T, CD19+ B and CD56+ NK cells were homogenously distributed in groups. In SSPE patients, CD4+CXCR3+ T cells, identifying Th1 subset, were significantly less frequent than in CON (2.9±1.8% vs. 6.4±3.2%, p=0.002), whereas CCR4 expressing CD4+ T cells (Th2) were not different between groups (11.0±6.7% vs. 11.6±6.4%).

The proportion of most probable Tregs, CD4+CD25high cells, was lower in SSPE patients than CON (1.0±4.1% vs. 1.6±2.8%, p= 0.043). CD8+ cells expressing inhibitory NKG2A+ receptor were also decreased (1.7±1.7% vs. 2.6±1.9%, p= 0.007), whereas NK cells expressing activating NKG2C were increased in SSPE patients (30.0±17.3% vs. 22.2±17.0%, p= 0.039).

The lower Th1 type cells implicate a decreased proinflammatory response and support our previous findings of lower proliferation as well as cytokine secretion in response to recall and MV antigens in SSPE. The lower presence of the inhibitory NK receptors on CD8+ cells and higher activating NK receptor in NK cells in SSPE may be caused by chronic stimulation of MV antigens leading to altered regulatory pathways in both cell groups.

The present work was supported by the Research Fund of Istanbul University (Project #: 192)

Keywords: SSPE, CXCR3, CCR4, Treg, NKG2A, NKG2C

P-055 *ABSTRACT REF.: 116*

INTRAVITREAL BONE MARROW DERIVED MESENCHYMAL STEM CELL THERAPY IN DIABETIC RETINOPATHY

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BACKGROUND: Intravitreal injection of mesenchymal stem cells has been reported to be effective in various retinal diseases. We studied the effects of stem cells on electroretinography and morphology of the diabetic retina.

METHODS: A total of 16 Wistar albino rats were randomly divided into diabetic (n=12) and control (n=4) groups. A single injection of streptozotocin was given to induce diabetes. Weights, blood sugar and full field ERGs (-25db,-10db,-5db, rod cone, oscillatory potential) were recorded weekly. After 12 weeks right eyes of diabetic rats were treated with intravitreal 20 µl (2x105 cells) bone marrow derived mesenchymal stem cells and left eyes with sham. At 1st week of treatment immunohistochemistry was conducted for vimentin, glial fibrillary acidic protein and rhodopsin in the retina.

RESULTS: Diabetic animals had a significant delay and decrease in the amplitude in a wave and b wave than normal (p=0.03, p=0.02). At first week mean amplitudes of a, b and oscillatory potential waves of stem cell treated group were found increased than of sham controls significantly. Immunohistochemistry revealed a significant increase in ganglion cell layer thickness (p=0.03) CONCLUSION: Bone marrow derived mesenchymal stem cells may integrate into the retina in diabetic retinopathy and have significant effects on improving the retinal signaling.

Keywords: bone marrow derived mesenchymal stem cell (BMD-MSC), diabetes, immunohistochemistry, retinopathy.

P-056 *ABSTRACT REF.: 134*

T CELL ACTIVITIES IN MYASTHENIA GRAVIS PATIENTS

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Acquired myasthenia gravis (MG) is a heterogeneous disease with respect to age at onset, thymic changes and presence of auto-antibodies (ab). The symptoms of MG are mainly mediated by pathogenic ab against the nicotinic acetylcholine receptor(AChR) in 85-90% of the patients (AChR-MG). A small and non-overlapping subgroup of MG patients has ab against muscle specific kinase (MuSK-MG) with different isotypes and different features to the main group. T cell related cytokine responses are compared in patients with AChR-MG and MuSK-MG to the healthy controls (CON) in this study.

Forthy-six AChR-MG and 23 MuSK-MG patients with generalized disease and 42 CON were included. Totally, 35-62% of MG patients were on immunosuppressive (IS) treatment. Cytokines with regulatory effects on ab production (IFNgamma, IL-6,IL-4,IL-13,IL-17A,IL-10 and IL-21) are evaluated in the plasma or by anti-CD3 stimulated or non-stimulated PBMC using ELISA or multiplex arrays. Gene expression levels (TBET,GATA3,RORC, FOXP3,BCL6, IFNG,IL10,IL17A,IL21,CD40L) in the positively isolated CD4+T cells were also determined by relative quantification.

IL-21 production of unstimulated as well as by anti-CD3 stimulated PBMC in MuSK-MG group was higher than CON(p=0.018 and p=0.001). Anti-CD3 stimulation induced higher IL-21 also in AChR-MG (p=0.021). In response to anti-CD3, IL-17A and IFNgamma production was increased in MuSK-MG compared CON(p=0.04 and p=0.002), whereas IL-13 levels were not different among all subgroups.

Expressions of master regulators and signature cytokines CD4+ T cells were similar in all subgroups. However, CD40L expression was lower in MG (p=0.001) and both in AChR-MG and MuSK-MG than CON(p=0.005 and p=0.004).

While plasma cytokine levels were not different among the subgroups, a decrease of IL-12p40 was observed in treated MG patients compared to CON and non-treated patients(p= 0.006 and 0.001). However, IL-10 levels were lower in un-treated MG patients compared to CON (p= 0.026). Similarly, in AChR-MG patients, spontaneous IL-10 production increased by use of IS(p=0.031) and the increased levels in cultures were higher than the CON(p=0.014). Effect of IS treatment has also been observed in increase of IL-6 production of AChR-MG compared to than non-treated patients(p=0.036).

With these findings, IL-21, IL-17A and IFNgamma may be regulating ab production in MuSK-MG by stimulated T cells. IL-10 and IL-6 seem to be up-regulated, while IL12p40 is suppressed in IS treated patients which implicate a mechanism of functional regulation by treatment in MG. Lower expression of co-stimulatory molecules by CD4+T cells may also contribute to this regulation in MG patients. This study is supported by TUBITAK (106S223 and 109S353).

Keywords: myasthenia gravis, cytokine, PBMC, T cell subsets

NOVEL DISCOVERIES IN THE INNATE IMMUNE SYSTEM

P-057 *ABSTRACT REF.: 002*

FEATURES OF TLR-2+, TLR-4+ AND NF-KB+ EXPRESSIVE LYMPHOCYTES OF THE INTESTINE UNDER STRESS

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The development of chronic social stress (CSS) accompanies with the changes of the intestinal microflora, which affects the level of signaling through pattern recognition receptors (PRR), such as Toll-like receptors (TLRs) and leads to activation of nuclear factor kappa-B (NF-κB). So, the aim of the research was to study the CSS influence on the features of TLR-2+-, TLR-4+- and NF-kB+-expressive lymphocytes in GALT Wistar rats. Researchers have been conducted on 70 rats (female) of Wistar line, which were divided on 3 experimental groups: control rats (group 1); rats, which were modeled CSS1 by means of three weeks social isolation and prolong psychoemotional influence (group2); rats, which having CSS 2 modeling by means of keeping animals in over populated cages with every day change of grouping (group 3). Structure of population of TLR2+-, TLR4+- and Nf-kB+-cells has been studied by the analysis of serial histological sections using the method of direct and indirect immunofluorescense with monoclonal antibodies (SantaCruzBiotechnology). We investigated lymphoid follicles (LF) and subepithelial zone (SZ) Peyer's patches (PP) and lymphocyte-filled villi (LFV). CSS development is accompanied with increase in total lymphocytes expressing TLR2 and 4 type GALT rats with the most pronounced in LFV (TLR2+-lymphocytes) and PP LFs (TLR4+-cells) led to an increase in the number of Nf-kB+-cells: in LFV a 1.8-2 fold ($p < 0.05$) in PP at the SZ -52-91% ($p < 0.05$) in PP LFs –for 89-92% ($p < 0.05$), and it is also influenced on the density of TLR2, TLR4, and the concentration of Nf-kB in immunopositive cells.

Keywords: stress, TLR, lymphocytes, GALT

P-058 *ABSTRACT REF.: 068*

IDENTIFICATION OF THE RELATIONSHIP BETWEEN NLRP7 PROTEIN AND IMMUNE PRIVILEGE

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NOD Like Receptor (NLR) proteins are cytoplasmic proteins, which take role in apoptosis and inflammation. NLRP7 is a primate specific protein and consist of PYRIN, NACHT and LRR domains. NLRP7 is found to has an oncogenic role in testicular seminoma and several cancer types. Additionally, mutations of NLRP7 cause recurrent hydatidiform moles, which result in stillbirths and abortions. In this project, our aim is to clarify the possible role of NLRP7 in immune privilege and inflammation.

As a result, we showed that NLRP7 forms an inflammasome by interacting with Caspase 1 and ASC and activates IL-1b. Co-IP studies also showed that NLRP7 has an interaction with Caspase 1, ASC and Caspase 5. We also identified that several different cell lines, especially in immune privilege sites have an increased NLRP7 expression. Each domain of NLRP7 and full length form of it were cloned and purified, particularly. Polyclonal antibodies against PYRIN and full length NLRP7 were produced. By using these antibodies, identification of NLRP7 activators in NLRP7 expressing cell lines with treated several pathogen associated molecular patterns (PAMP), danger associated molecular patterns (DAMP), heat killed bacteria and also endogenous CO-IP studies are still ongoing studies.

Our results showed that NLRP7 forms an inflammasome to activate the IL-1b by binding to ASC and Caspase-1. NLRP7 directly interacts with ASC, Caspase-1, Caspase-5 and certain PAMPs and DAMPs stimulate NLRP7 expression.

Keywords: Innate immunity, inflammasome, NOD like receptors, NLRP7.

P-059 *ABSTRACT REF.: 066]*

IMMUNOTHERAPEUTIC CPG OLIGODEOXYNUCLEOTIDE/ CATIONIC PEPTIDE NANORINGS AS PLASMACYTOID DENDRITIC CELL STIMULATORS

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Type I interferons play important roles in regulating innate and adaptive immune responses with potential applications as anti-viral/anti-cancer agents. One such type I interferon inducer, D-type CpG ODN, remained clinically unexplored owing to formation of uncontrollable product aggregation that complicates their manufacturing. Herein, we aimed to convert a conventional K-type CpG ODN suitable for clinical use but devoid of interferon-alpha stimulating activity into a potent type I interferon inducer by complexing it with the cationic peptide Tat(47-57) into nanometer sized nanorings. The nanorings improved nuclease resistance, enhanced cellular uptake and stimulated a robust IFN alpha from plasmacytoid dendritic cells. Confocal studies confirmed that the nanorings targeted the ODN to transferrin positive early endosomes, a subcellular distribution pattern consistent with efficient IFN alpha production. In vivo, K/Tat nanorings proved to be potent vaccine adjuvants and induced Th1-dominated long-term immune response in mice vaccinated with a 5-X lower dose of a commercially available inactivated viral vaccine against the FMDV. Therapeutic vaccination of C57BL/6 mice bearing ovalbumin (OVA)-expressing EG.7 thymoma tumors with OVA plus K/Tat nanorings generated superior anti-tumor immunity when compared to mice treated with OVA plus K-ODN. The in vivo vaccine adjuvant activity of the nanorings was similar to D ODN and was severely impaired in mice depleted of pDCs, suggesting that pDC activation and their type I IFN production is critical for the vaccine adjuvant activity of the nanorings. In conclusion, these immunostimulatory nanorings are effective D-ODN surrogates and could prove to be of value as anti-viral or anti-cancer agents and vaccine adjuvants in the clinic.

Keywords: CpG ODN, TLR 9, IFN alpha, Tat peptide, nanoring

P-060 *ABSTRACT REF.: 061*

SCAVENGER-RECEPTOR MEDIATED AND CLATHRIN-DEPENDENT ENDOCYTOSIS REGULATE INTERNALIZATION OF EXTRACELLULAR VESICLES BY IMMUNE CELLS

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Microparticles are member of naturally occurring, membranous nanovesicles collectively known as extracellular vesicles (EVs) function as intercellular communication vectors. Upon internalization, they can regulate cell physiology as well as mediate immune response. In this study we aimed to explore microparticle uptake mechanism of macrophages. EVs were isolated from macrophage like (RAW264.7), megakaryocyte, (Meg01), T-cell, (E.G7-OVA) and fibroblast (NIH3T3) cell line supernatants by differential centrifugation, filtration and ultracentrifugation. They were stained with lipophilic chromophores and incubated with RAW264.7 cells at different time intervals. Experiments were conducted in the presence or absence of different endocytosis inhibitors, such as sodium azide, ammonium chloride and sucrose. In some experiments, scavenger receptor antagonists (i.e. fucoidan and dextran sulfate) were used either as such or along with above-mentioned inhibitors. Cellular binding, uptake and overall internalization levels of EVs were analyzed with flow cytometry. In some experiments, HEK293T cells were transiently transfected with RAB 5, 9, 11 and CD63 plasmids either expressing GFP, RFP or YFP proteins, and incubated with EVs to track time course sub-cellular fate post- internalization by Confocal Microscopy. It was revealed that EV origin influence binding and internalization kinetics and levels by macrophages. Formation of clathrin coated-pits on the cell surface is critically contributing the internalization of different EVs within target cell. When cells were preincubated either with fucoidan or with dextran sulfate, and incubated with EVs for an hour 30-40% reduction in EV internalization was observed. Initial confocal studies implicated that upon internalization EVs appears to be co-localized in CD63+ compartments. RAB protein association at early phases of internalization seems to be unrelated. Naturally occurring EVs are known to involve in regulation of immunomodulation; and their number and composition are altered in many different diseases. Further, cell-derived EVs can be used as a drug-delivery system and for vaccination or anti-cancer therapy purposes. Therefore, it is important to understand entry and accumulation mechanisms of EVs as well as their fates upon internalization. In that respect, specific to macrophages we identified two new pathways responsible for EV binding and uptake. This finding may help to control EV mediated therapy against diseases.

Keywords: extracellular vesicles, clathrin-coat mediated endocytosis, scavenger receptor, macrophages

P-061 *ABSTRACT REF.: 069*

NLRC3 IS A REGULATOR OF CRYOPYRIN INFLAMMASOME

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NLR proteins are cytoplasmic receptors which recognize pathogenic molecules. The NLRC3 protein, a novel member of this family, was shown to inhibit the NFkB pathway through TRAF6 ubiquitination and its expression was inversely correlated with T-cell activation. However, the role of NLRC3 in cytokine secretion such as IL-1β is still unknown.

In order to investigate whether NLRC3 has a role in IL-1β secretion by itself and to determine if it affects Cryopyrin inflammasome activity, overexpression studies, ASC speck formation assays and induction of Cryopyrin inflammasome in WT and NLRC3 knock-down cells were performed. When overexpressed in HEK293FT cells together with ASC, Caspase-1 and IL-1β, NLRC3 slightly induced IL-1β secretion in a dose-dependent manner, however the level of secretion was lower compared to Cryopyrin. Moreover, transfection of NLRC3 in HEK293FT-ASC-GFP stable lines did not induce ASC speck formation significantly. Interestingly, whereas Cryopyrin induced ASC speck formation highly, addition of NLRC3 decreased ASC speck number. Furthermore, IL-1β secretion significantly increased in NLRC3 knocked-down stable THP-1 lines.

In summary, NLRC3 had no significant effect on IL-1β secretion on its own but inhibited IL-1β secretion and ASC speck formation induced by Cryopyrin. Thus, NLRC3 may have an inhibitory role in inflammation as it appears to compete with Cryopyrin for inflammasome activation.

Keywords: NLRC3, Cryopyrin, IL-1β, Inflammation

P-062 *ABSTRACT REF.: 034*

EFFECTS OF IL-33 ON NO PRODUCTION AND PKC-CAMP-NFKB SIGNALLING PATHWAY IN J774.1 MACROPHAGES

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Interleukin-33 (IL-33), a novel member of IL-1 family cytokines, is a potent proinflammatory cytokine that stimulates the production of Th2-associated cytokines. Whereas IL-1β and IL-18 which are the members of this family promote Th1 associated type I immune response, IL-33 induces Th2 polarisation. In addition, IL-33 transmits its signal as the same signalling pathway as the other IL-1-related cytokines. IL-33 cytokine plays role in pathogenesis of some diseases. It has a protective effect in atherosclerosis and the role of pathogenesis of IBD, asthma, RA, contact dermatitis and psoriasis. Signalling pathways is important to determine appropriate hypothesis in studies of therapeutic agents related to signalling pathways. This study is aimed to enlighten the effect of IL-33 on intracellular signalling pathways, in particular on PKC and cAMP pathways, in macrophage cell line, J774.1.

METHODS: mrIL-33 was added into cells stimulated by IFN-γ plus LPS or unstimulated. Then, protein kinase C activity, levels of cAMP and NF-κB activities was measured by ELISA. RESULTS: When added to naive macrophages, IL-33 decreased cAMP accumulation, enhanced PKC activation and caused to increase NF-κB activation at the first hour, then to diminish. When IL-33 added to IFNγ plus LPS-treated macrophages, cAMP production and the translocation to the membrane of PKC were increased. IL-33 caused to reduce NF-κB activation in IFNγ plus LPS-treated macrophages. The same results of IL-33 were obtained in TNF-α stimulated macrophages. In parallel experimental design, NO production was measured by griess reaction. CONCLUSION: Due to the mechanism, IL-33 keeps macrophages in priming state in prior to induction with IFN-γ plus LPS or TNFα and represses the severe inflammation. The present study establishes for the first time that cAMP, PKC and NF-κB are become effective on the IL-33 signalling pathway. IL-33 is significantly diminished nitrite levels in macrophages pretreated with IFNγ+LPS in time and dose dependent manner. This study is a pioneering study for observation of the effect of IL-33 in intracellular signalling pathways of macrophages. Then, it will be going through the proper channels to understand effective mechanisms of IL-33, as a therapeutic target of some diseases such as asthma.

Keywords: interleukin-33, signalling, nitric oxide, macrophage, cAMP, PKC, NFkB pathway

P-063 *ABSTRACT REF.: 035*

EFFECTS OF IL-33 ON MACROPHAGE PHAGOCYTOSIS AND NITRIC OXIDE PRODUCTION IN DIFFERENT MICROENVIRONMENTAL CONDITIONS

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Interleukin-33 is classified as a new member of interleukin-1 family cytokines. Whereas IL-1 and IL-18 promote Th1 associated immune response, IL-33 induces Th2 polarisation. So far, induction or inhibition of NO production by many other cytokines, such as IL-4, IL-13, IL-10 and TNFα, have been reported.Our study was aimed to explore the effect of IL-33 in macrophages.

METHODS: IL-33 was added into cell cultures of J774.1 stimulated or unstimulated with IFN-γ plus LPS. Macrophages were pretreated with IFNγ+LPS for 24h (M1), IL-4 (10ng/ml)+IL-13 (10ng/ml) for 24h (M2a) and IL-10 (100ng/ml) for 24h (M2c), then administered different doses of IL-33 for 18h. Having applied, NO production was measured by Griess reaction in 24h and 48h. Chemokine and chemokine receptors were assessed by IFA staining (direct or indirect) and ELISA. CCR7, CCR2 and CXCR1 (CD181) expression, CCL22 and IP10 production were determined. Preliminary phagocytosis experiments were performed with E.coli antigen coated and fluorescently labelled polystyrene beads. Then FITC labelled and heat inactivated bacteria was used for phagocytosis assays following exposure of cells to different stimulation as described above. Fluorescence of extracellular particles was quenched by adding Trypan blue 2%. Phagocytosis was observed by using fluorescent microscopy, and NO was synchronically measured. In addition, we added A. baumannii bacteria into cultures of polarized macrophages (M1, M2a, M2c) with or without IL-33.

RESULTS: We found that IL-33 did not affect NO synthesis in unstimulated J774.1 cells. IL-33 diminished nitrite levels in macrophages pretreated with IFNγ+LPS in time and dose dependent manner. IL-33 increased nitrite levels in macrophages stimulated with IL-4+IL-13. Finally, IL-33 did not change nitrite levels in macrophages stimulated with IL-10. It was seen that stimulated or unstimulated cells expressed CCR2. IFNγ+LPS and LPS stimulation led to CCR7 receptor expression. IL-33 had no effect on the induction CCR7 expression on J774.1 cells. While unstimulated or stimulated cells by high dose LPS were found to stain negative for CXCR1, it was observed that IL-33 caused to CD181 receptor expression and CCL22 production. When added IL-33 alone into unstimulated cells, fluorometric and flow cytometric analysis showed that IL-33 caused to increase in phagocytosis response of macrophages. IL33 also increased phagocytosis ratio in IFNγ+LPS stimulated cells. While IL-4+IL-13 was decreasing phagocytosis ratio, IL-33 led to raise phagocytosis capacity in IL-4+IL-13 and IL-10 stimulated macrophages.

CONCLUSION: The different inflammatory conditions have modified the effects of IL-33 in J774.1 macrophages

Keywords: interleukin-33, macrophage, nitric oxide, phagocytosis

P-064 *ABSTRACT REF.: 071*

CHARACTERIZATION OF NLRP13 IN INFLAMMASOME ACTIVITY AND IMMUNE PRIVILIGE

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Nod-like receptors are members of a protein family that sense infection, cellular damage and pathogens. NLRs consist of N terminal interaction domain (PYRIN), central oligomerization domain (NACHT/NOD) and leucine rich repeat domain (LRR) at the C terminus. The inflammasomes are multiprotein complexes consisting of caspase 1, ASC, NLR and sometimes caspase 5. Depending on the activator, components of the inflammasome assemble and maturation of inflammatory cytokines such as IL-1β and IL-18 occurs. We found that NLRP13 can interact with caspase 1, ASC and IL-1β. However, the effect of NLRP13 on Caspase 1 cleavage, IL-1β expression and secretion are not well known. Our preliminary results show that NLRP13 negatively regulates IL-1β maturation and secretion. Immune privilige sites such as brain and placenta are suggested to be tissues with NLRP13 expression and this may be a clue for the immune suppressive role of NLRP13 in these tissues. We produced NLRP13 antibodies for endogenous experiments and made NLRP13 sh-knockdown cell lines to understand possible roles of NLRP13 in inflammasome activity and immune privilege

Keywords: Innate immunity, immune privilige, inflammasome

P-065 *ABSTRACT REF.: 110*

THE EFFECTS OF ATOVAQUONE AND ASTRAGALUS COMBINATION ON MICE MODELS WITH ACUTE TOXOPLASMOSIS AND THEIR IL-2, IL-12, INF-γ LEVELS

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Toxoplasmosis is the cause of serious clinical conditions in immunspressed patients. Atovaquone is an antiprotozoal with little side effect against toxoplasmosis. Astragalus membranaceus root extract (AmE) has been showed as phagocytic activity enhancer, immunomodulator and immunostimulant agent. The aims of this study were to investigate the effectiveness of atovaquone alone and in combination with AmE and to determine the levels of IL-2, IL-12 and INF-γ in experimentally infected mice with T.gondii.

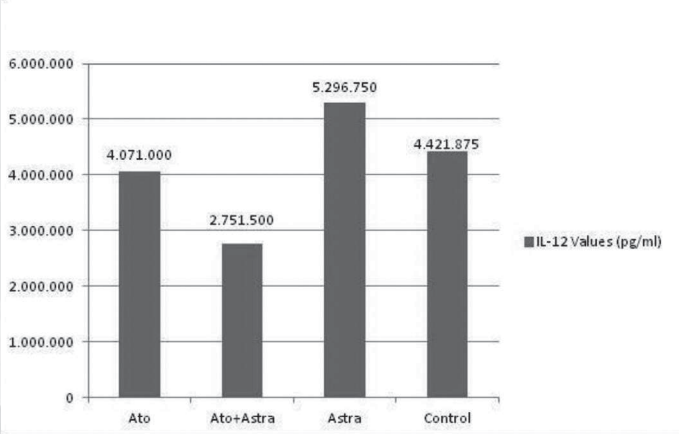
For this purpose, four experimental groups, each consisting of eight BALB/c mice, were formed with the approval of Ethics Committee for the Animal Experiments. All the mice were infected with 0,5 ml of suspension containing 2x104 ml trophozoite prepared from T.gondii RH strain by intraperitoneal injection. Twenty-four hours after the infection, atovaquone (100 mg/kg/day) was given to atovaquone group, AmE (0,075 mg/gr) to astragalus group and atovaquone (100 mg/kg/day) plus AmE(0,075 mg/gr) to Atovaquone+Astragalus (Ato+Astra) group by oral gavage. The fourth group, which was the control group, was all infected but untreated. The above administration was carried out for seven days. On the 8 th day, under anaesthesia,1 ml normal saline was

given into the peritoneum, drawn back and the number of trophozoites in 1 ml of peritoneal fluid was determined by counting them on the Thoma slide. Moreover, by drawing the heart blood IL-2, IL-12, INF-γ levels were determined in serum sample by ELISA method. The number of trophozoites in Ato+Astra group was found significantly lower than the number of trophozoites in other three groups (P<0,05). The number of trophozoites in atovaquone and astragalus groups were found significantly lower than the number of trophozoites in control goup (P<0,05). There was a significant increase in IL-2 levels of astragalus group compared with other three groups, in addition when IL-2 levels of Ato+Astra group were compared with ones in other three groups, a significant decrease was noticed (P<0,05). There was a certain increase in IL-12 levels of atovaquone, astragalus and control groups compared to Ato+Astra group's (P<0,05). A significant increase was found in INF-γ levels in atovaquone and Ato+Astra groups compared with control group's (P<0,05).

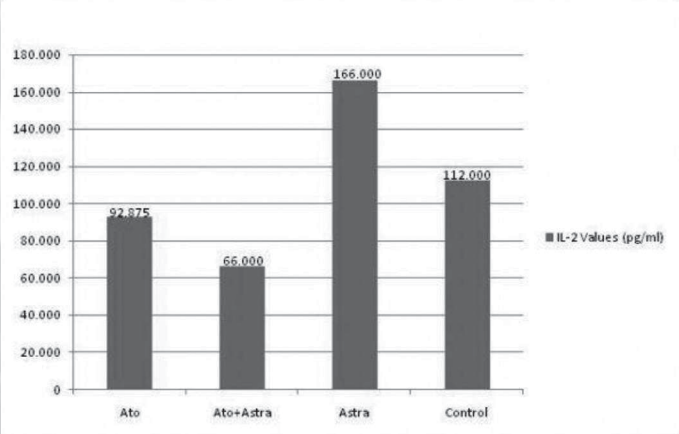
Within the reach of our literature survey, this study is the first research in which the effectiveness of the combination of atovaquone and AmE was investigated in the treatment of acute toxoplasmosis. The results of our study suggested that there might be synergy between atovaquone and AmE in the treatment of acute toxoplasmosis.

Keywords: Astragalus membranaceus, Atovaquone, IL-2, IL-12, INF-γ, Toxoplasma gondii

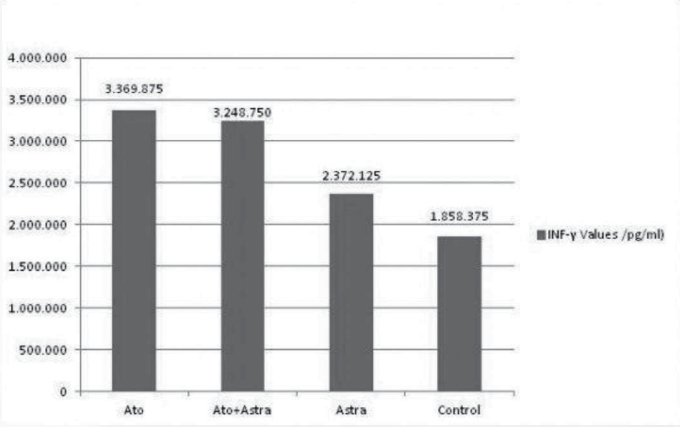
Average Values of Experimental Groups IL-12 Levels



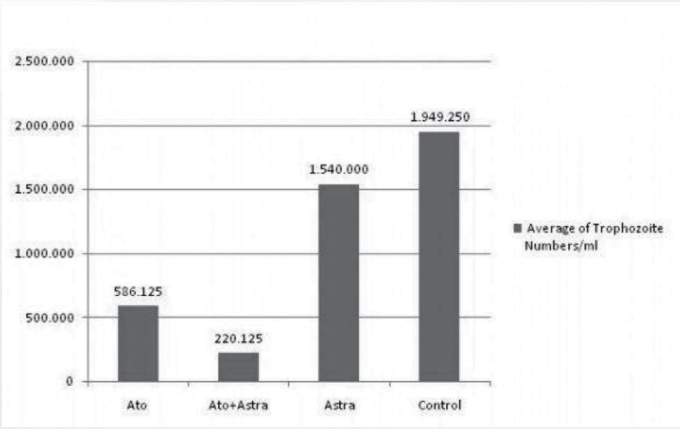
Average Values of Experimental Groups IL-2 Levels



Average Values of Experimental Groups INF-γ Levels



Average Values of Experimental Groups Trophozoite Numbers



Statistical Values of IL-12 (pg / ml)

Compared Group	Other Groups	Difference Between Groups	Standard Deviation	P Values
ATO	Ato+Astra Astra K	1319,500* -1225,750* -350,875	±468,312	0,009 0,014 0,460
ATO+ASTRA	Ato Astra K	-1319,500* -2545,250* -1670,375*	±468,312	0,009 0,000 0,001
ASTRA	Ato Ato+Astra K	1225,750* 2545,250* 874,875	±468,312	0,014 0,000 0,072
K	Ato Ato+Astra Astra	350,875 1670,375* -874,875	±468,312	0,460 0,001 0,072

** Statistically significant (P <0.05) difference between the groups values.*

Statistical Values of IL-2 (pg / ml)

Compared Group	Other Groups	Difference Between Groups	Standard Deviation	P Values
ATO	Ato+Astra Astra K	26,875* -73,125* -19,125	±11,846	0,031 0,000 0,118
ATO+ASTRA	Ato Astra K	-26,875* -100,000* -46,000*	±11,846	0,031 0,000 0,001
ASTRA	Ato Ato+Astra K	73,125* 100,000* 54,000*	±11,846	0,000 0,000 0,000
K	Ato Ato+Astra Astra	19,125 46,000* -54,000*	±11,846	0,118 0,001 0,000

** Statistically significant (P <0.05) difference between the groups values*

Statistical Values of INF- γ (pg / ml)

Compared Group	Other Groups	Difference Between Groups	Standard Deviation	P Values
ATO	Ato+Astra Astra K	121,125 997,750 1511,500*	±504,760	0,812 0,058 0,006
ATO+ASTRA	Ato Astra K	-121,125 876,625 1390,375*	±504,760	0,812 0,093 0,010
ASTRA	Ato Ato+Astra K	-997,750 -876,625 513,750	±504,760	0,058 0,093 0,317
K	Ato Ato+Astra Astra	-1511,500* -1390,375* -513,750	±504,760	0,006 0,010 0,317

** Statistically significant (P <0.05) difference between the groups values.*

Statistical Values of Trophozoite Number (Trophozoite/ml)

Compared Group	Other Groups	Difference Between Groups	Standard Deviation	P Values
ATO	Ato+Astra Astra K	366,000* -953,875* -1363,125*	±111,515	0,003 0,000 0,000
ATO+ASTRA	Ato Astra K	-366,000* -1319,875* -1729,125*	±111,515	0,003 0,000 0,000
ASTRA	Ato Ato+Astra K	953,875* 1319,875* -409,250*	±111,515	0,000 0,000 0,001
K	Ato Ato+Astra Astra	1363,125* 1729,125* 409,250*	±111,515	0,000 0,000 0,001

** Statistically significant (P <0.05) difference between the groups values*

P-066 *ABSTRACT REF.: 120*

FLOW CYTOMETRIC DETERMINATION OF VITAMIN D RECEPTOR EXPRESSION IN HUMAN IMMUNE CELLS

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Vitamin D (VitD) plays roles not only in skeletal homeostasis, but also in immunomodulation, through binding to vitamin D receptor (VDR), a member of the nuclear receptor superfamily, in cells of the immune system. Currently, VDR expression in these cells can only be shown semi quantitatively by PCR analysis and western blotting. In this study, we describe a new multiparametric flow cytometric method for direct quantitation of VDR in monocytes and lymphocytes. A rat monoclonal antibody recognizing both monomeric and dimeric forms of the receptor was optimized using human peripheral blood mononuclear cells. Percentages of VDR in peripheral monocytes, T and B cells in healthy adults were shown as 42.3 ± 12.5%, 25.4 ± 12.4% and 2.5 ± 2.0%, respectively (n = 20). In addition, we also determined that VDR expression in monocytes of severely VitD-deficient chronic kidney disease patients (n = 10) is markedly increased compared to healthy controls (62.1 ± 15.0% vs. 42.3 ± 12.5%, p = 0,017). In conclusion, herein we describe the first multi parametric flow cytometric protocol in literature to quantitatively determine VDR expression in immune cells of humans, which should facilitate future studies on VitD and its receptor in immune system.

Keywords: Vitamin D (VitD), Vitamin D Receptor (VDR), monocyte, lymphocyte, flow cytometric method

REGULATORY CELLS OF IMMUNE SYSTEM

P-067 *ABSTRACT REF.: 140*

ABBERANT EXPRESSIONS OF CD68 AND CD45 IN MEDIASTINAL ADIPOSE TISSUE ARE ASSOCIATED WITH THE INCREASED SECRETION OF THE PROINFLAMMATORY CYTOKINES IN OBESE CORONARY ARTERY DISEASE

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Increasing evidence highlights the role of adipose tissue in the development of a systemic inflammatory state that contributes to obesity-associated vasculopathy and cardiovascular risk. Circulating mediators of inflammation participate in the mechanisms of vascular insult and atheromatous change, and many of these inflammatory proteins are secreted directly from adipocytes and adipose tissue-derived macrophages. The aim of this study was to investigate the contributory role of inflammation in the development of cardiac adiposity in coronary artery disease (CAD).Along with the somatic variables, the proinflammatory markers; hsCRP, interleukin-6 (IL-6),

interleukin-18 (IL-18), TNF-α and adiponectin as well as erythrocyte sedimentation rates and leucocytes count in sera were measured and mRNA expression levels of inflammatory markers C3, CD45 and CD68 were studied in three different adipose tissues of the 37 obese patients with CAD underwent elective coronary artery bypass grafting (CABG) and of 23 obese patients with without CAD underwent valvuloplasty surgery. The level of hs-CRP, IL-6, IL-18, TNF-α, ESR and leucocyte count in sera were measured by ELISA and calorimetric biochemical assays. mRNA expression levels of inflammatory markers C3, CD45 and CD68 were studied by QRT-PCR. Our result showed that all of these proinflammatory markers, except for CRP, were found to be significantly higher in obese patients with CAD compared to obese patients without CAD. C3, CD45 and CD68 mRNA expressions levels were present in epicardial adipose tissue (EAT), mediastinal adipose tissue (MAT) and subcutaneous adipose tissue (SAT) of obese patients with CAD and obese patients without CAD. The CD68 mRNA expression levels were found to be significantly increased in EAT, MAT, SAT of obese patients with CAD compared to obese patients without CAD ($p<0.05$). In MAT of obese patients CAD group, CD68 and CD45 mRNA expressions levels were found to be higher compared to EAT and SAT (nearly ten fold and five fold, respectively). The C3 mRNA expressions levels were found to be significantly increased in MAT of obese patients with CAD compared to obese patients without CAD groups ($p<0.001$).

Our results demonstrate that IL-6, IL-18, TNF-α, ESR could be potential markers for CAD. High levels of CD68 and CD45 in MAT tissue indicate well the macrophage infiltration to this tissue. We conclude that MAT tissue in CAD with obese patients might well trigger macrophage polarization to the M1 direction which would result in the secretion of the proinflammatory cytokines playing a major role in CAD pathogenesis.

Keywords: mediators of inflammation, coronary artery disease (CAD),macrophages

P-068 *ABSTRACT REF.: 093*

INTRACELLULAR MECHANISMS OF IL-10 EXPRESSION FROM HELICOBACTER-ACTIVATED REGULATORY B CELLS

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Helicobacter pylori (H.pylori) is a microaerophilic bacterium that contributes to development of gastric malignancies that may lead to gastric cancer. Although nearly half of the world's population is infected with H.pylori, majority of infected individuals are asymptomatic. Interleukin-10 (IL-10), an anti-inflammatory cytokine, represses immune response against pathogens and attenuates damage to host, thereby prevents autoimmunity. Regulatory T (Tregs) and B (Bregs) cell subsets are known to secrete IL-10. Our previous research indicated that Bregs have suppressive and immune regulatory role in mouse models of Helicobacter infection. Helicobacter-activated regulatory B cells (Hact-Bregs) secrete IL-10 through Toll-like receptor-2 (TLR-2) signaling. Recently, it has been reported that TLR-2-mediated IL-10 secretion is maintained through MAPK/ERK (mitogen-activated protein kinase/extracellular signal-regulated kinase) signaling pathway in macrophages and dendritic cells. However, the intrinsic signal transduction pathways responsible for expression and secretion of IL-10 in Helicobacter-activated Bregs have not been shown. For that reason, we aim to analyze MAPK/ERK signaling pathways in murine splenic naïve B cells, splenic B cells treated with Helicobacter sonicate and also IL-10+ Hact-Bregs which are magnetically separated from Helicobacter-treated B cells. MAPK/ERK signaling pathway activation requires phosphorylation of two protein kinases, p38 MAPK and p44/42 MAPK (ERK-1/2), respectively. Western blotting was used to detect levels of both total and phosphorylated forms of these kinases. Importance of our research underlies in its contribution to comprehend the intracellular signaling pathways of IL-10 expression and secretion in Hact-Bregs for the first time. In conclusion, our work suggests that IL-10 expression is maintained through phosphorylation of p44/42 MAPK (ERK-1/2), but not p38 MAPK in IL-10+ Hact-Bregs.

Keywords: IL-10, Helicobacter, Regulatory B cell, MAPK/ERK

P-069 *ABSTRACT REF.: 117*

STEM CELLS FROM HUMAN EXFOLIATED DECIDUOUS TEETH SUPPRESS IN VITRO PROLIFERATION OF LYMPHOCYTE CELLS FROM ASTHMA PATIENTS

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OBJECTIVE: In this study, we aimed to investigate the effect of stem cells from human exfoliated deciduous teeth on lymphocytes proliferation isolated from asthma patients.

METHOD: Human exfoliated deciduous teeth (SHED) from 8-14 years old childrens were obtained from Marmara University Faculty of Dentistry Oral and Maxillofacial Surgery. Stem SHED were isolated, characterizated and differentiated. Asthmatic patients (n=10) and control (n=5) bloods were obtained from 8-14 years old children from Marmara University Education and Research Hospital Pendik department of Pediatric Allergy-Immunology. PBMC, were isolated and labeled with CFSE, then they stimulated with antiCD3- antiCD2-antiCD28 with and without SHED. After 3 days of culturing lymphocyte proliferation were analiyzed by flow cytometry.

RESULTS: According to the results in control grup, we don`t find any changes in the rate of lymphocyte proliferation in the presence and absence of SHED (without SHED %29±29, with SHED %29±29). SHED were suppression the proliferation of lymphocytes from asthmatic patients compared non SHED environment (without SHED: %26±13, with SHED %11±10).

CONCLUSION: Comparing with control group, stem cells from human exfoliated deciduous teeth suppress in vitro proliferation of lymphocyte cells from asthma patients.

Keywords: Asthma, Proliferation, Stem cells from human exfoliated deciduous teeth

TRANSPLANTATION IMMUNOLOGY

P-070 *ABSTRACT REF.: 128*

HLA ALLELE FREGUENCIES IN THE SOUTESTERN, EASTERN AND MEDITERRANEAN REGION OF TURKEY

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INTRODUCTION: It is important to known HLA frequencies in population for HSCT and it is differ between populations. These differences are reflected in matching probabilities of recipient and potential donors.This study the first to be carried out with such mass screening of Turkey. OBJECTIVES: The aim of the retrospective study was to investigate HLA class-I and class-II allele frequencies in our region.

MATERIALS-METHODS: Tissue typing for HLA class I (-A, -B,-C) and class-II (-DRB1,-DQB1) in 3142 patients (n:1345) and donors (n:1797) who applied Baskent University Adana Research and Medical Center (between 2010 and 2013) for renal transplantation or hematopoietic stem cell transplantation, were studied using sequence-specific primers (SSP) and/or sequence-specific oligonucleotides (SSO).

RESULTS: Our group consists of female 45.1% (n:1416) and male 54.9% (n:1726) patients and donors and their mean of age is 40,1±16,1. A total of 21 HLA-A, 31 HLA-B, 14 HLA-C, 13 HLA-DRB1 and 5 HLA-DQ alleles were identified. The most frequent HLA alleles were HLA-A*02 (32.9%), HLA-A*24(27.0%), A*03 (24.0%), HLA-B alleles were HLA-B*35 (31.6%), HLA-B*51 (23.0%), HLA-B*44 (11.5%), HLA-B*18 (10.1%), HLA-C*04 (15.64%), HLA-C*07 (13.85 %), HLA-C*12 (12.17%) and for class-II; HLA-DRB1*11 (44.9 %), HLA-DRB1*04 (28.8%), and HLA-DRB1*15 (22.8%), HLA-DQB1*03 (46.0 %) and HLA-DQB1*05 (23.3 %) We did not find any correlation between HLA allel frequencies and sex (p>0.005), and donor or patients (p>0.005).

CONCLUSION: Our study results show that there is no correlation between patients and donors. So we can accept our results as the real HLA frequecies independent from hematological disease and end stage renal failure. According to our literature search HLA-A*02, HLA-A*24, HLA-A*01 and HLA-B*35, HLA-B*51, HLA-B*44 are the most frequent allel in İstanbul city region (Uyar et al). The other study is HLA-A*02, HLA-A*24, HLA-A*11 and HLA-B*35, HLA-B*51, HLA-B*44 HLA-DRB1*11, HLA-DRB1*04, and HLA-DRB1*13 are the most frequent allel in Eastern Anatolian region of Turkey (Kayhan et al). When we compare our results with the others, except some small differences, HLA frequencies results were similar to each other although they are from different regions of Turkey.

Keywords: HLA, allele, frequencies

P-071 *ABSTRACT REF.: 114*

REGAINED T AND B CELL PROLIFERATIVE CAPACITIES IN PEDIATRIC IMMUNE DEFICIENCY PATIENTS AFTER BONE MARROW TRANSPLANTATION

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Primary immune deficiencies are a group of rare disorders which mainly affect pediatric patients. Deficient cells are main reason for immune disfunction, leading to disposition to life-treating infections. Bone marrow transplantation is a therapy option for this group of disorders. Defining time points for vaccination is an important issue for transplant recipients and perfect timing is essential for proper vaccine responses. Proliferative capacity is an important parameter for estimation of cell functions. Carboxy fluorescein succinimidyl diester (CFSE) dilution is a flow cytometric method, which is widely used in measurement of proliferative capacity and can inform about the proliferating cell subsets.

This study aimed to investigate proliferative capacities of pediatric bone marrow transplant recipients with primary immune deficiency diseases.

Peripheral blood mononuclear cells (PBMC) were isolated from fresh heparinized blood samples of immune deficiency patients after 1 year of transplantation, and also from healthy subjects. Cells were stained with CFSE and stimulated with anti-CD2, -CD3 and -CD28 (CD-mix) as well as phytohemaglutinin (PHA), both of which act as polyclonal activators and CD4+ and CD19+ lymphocyte proliferations were investigated on day +5 of cell culture by flow cytometry. The results of this study showed normal proliferation of T and B cells after 1 year of transplantation in 19 out of 25 patients. Spontaneous as well as PHA- and CD-Mix induced proliferation percentages of total PBMC, CD4+ T- and CD19+ B-cells of transplant recipients were shown to be similar to healthy controls.

Our results clearly demonstrate re-gaining of proliferative capacity in bone-marrow transplant recipient primary immune deficiency patients, which makes flow cytometric proliferation analysis by CFSE dilution a perfect tool for determining success of bone marrow transplant in immunological aspect.

Keywords: Immune deficiency, proliferation, flow cytometry, bone marrow transplantation

TUMOR IMMUNOLOGY & MOLECULAR TARGETS

P-072 *ABSTRACT REF.: 048*

MUTATING THE P53 GENE USING TALEN PROTEINS

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TALE (Transcription activator-like effector) proteins contain a DNA-binding domain which is composed of tandemly arranged 33-35 amino acid repeats; each of these repeats bind specifically to one DNA base through the 12th and 13th residues called repeat variable di-residues (RVD). Designed TALEN proteins are genome editing tools generated by fusing TALE repeats to the catalytic domain of the FokI DNA restriction enzyme. Expression of two TALENs target and generate double stranded breaks in any desired region in genomic DNA. We used TALENs against the start codon of the p47 alternative splice variant of the p53 gene to generate p47 knockout cells. p47 is an N-terminal truncated isoform of p53 which has an important role in p53 localization and regulation by MDM2.We found that human colon cancer HCT116 p53 -/- knock out cells still retain the expression of p47.To remove this expression, we transfected these cells with TALENs against p47. To detect mutations in the targeted gene locus, we performed RFLP analysis with the BtsCI restriction enzyme, which recognizes the linker region between the two TALEN binding sites in the p47 gene. We identified HCT116 cells with various deletions in the p47 coding region and generated single cell clones of these mutants.Biochemical experiments with these cell lines are ongoing. These p47 knock out cell lines will allow us to identify the function of the p47 alternative splice variant of the p53 gene.

Keywords: TALEN, p53, genome editing, p47

P-073 *ABSTRACT REF.: 067*

MUTATION OF THE IL-7R GENE CONTROL REGIONS USING TALEN PROTEINS

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Nuclear factor kappa B (NF-kB) is one of the most important transcription factors in cell signaling influencing many pathways that lead to cell survival, proliferation and differentiation. Interleukin 7 receptor (IL-7R) is an essential element in T lymphopoiesis and IL-7 is the key signaling factor in early thymocytes. IL-7R is composed of an alpha chain (also known as CD127) and IL-2R gamma chain.

An NF-kB binding motif was identified in the evolutionary conserved region 3 (ECR3) of the IL-7Ralpha containing a putative transcriptional enhancer, but a direct relationship between NF-kB and IL-7R expression is missing in the literature. To identify the functional significance of this NF-kB binding motif, we mutated it in murine T lymphocyte cell lines using transcription activator-like effector nuclease (TALEN) genome editing technology. TALENs consist of a sequence-specific TALE DNA binding domain fused to a FokI nuclease domain which generates DNA double stranded breaks in targeted gene loci.

We hypothesized that mutation of this NF-kB motif would down-regulate IL-7R expression in the murine RLM11 CD4+ thymoma cell line that is naturally IL-7Rhigh. We used restriction fragment length polymorphism (RFLP) assay to detect the presence of mutations in the targeted region and we analyzed IL-7R expression by flow cytometry of RLM11 cells. We sequenced PCR amplicons of mutated, IL-7Rlow RLM11 cells and identified TALEN-induced mutations resulting in 8bp and 16bp deletions near or containing the NF-kB binding site. These mutant sites have been cloned into luciferase constructs and their activities have been found to be significantly lower than that of full length enhancer region.

Our studies so far have shown that NF-kB is a regulator of IL-7Ralpha expression and TALEN-induced mutagenesis is a reliable, target-specific method that can be used to manipulate gene expression.

Keywords: Interleukin-7 receptor, NF-kB, TALEN

P-074 *ABSTRACT REF.: 135*

MODULATION OF ICOS LIGAND (ICOSLG) EXPRESSION ON HL-60 MYELOID LEUKEMIA CELLS-INDUCED WITH IFN-GAMMA

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INTRODUCTION: ICOSLG (B7-H2), the ligand for inducible costimulator (ICOS), is important for the production of activation signals that complement the main T cell costimulatory pathway. Even though ICOSLG mRNA can be promiscuously found in many tissues, its surface expression is primarily detected on professional antigen presenting cells. As of myeloid cell origin, leukemic blasts can also be positive for ICOSLG. This preliminary study aims to identify the expression kinetics of ICOSLG in myeloid leukemia cells that were directed towards monocytic differentiation by IFN-gamma treatment.

METHODS: HL-60 myeloid leukemia cell line was used as a model for myeloid differentiation. Cells were stimulated with 200 U/ml IFN-gamma for 0, 4, 8, 16, 32, 64, and 128 hours. The changes in ICOSLG and CD14 expression were measured with flow cytometry. Following RNA isolation and DNase treatment, cDNA was synthesized and relative change in ICOSLG mRNA levels was determined with real-time RT-PCR assays. B-actin expression was used as house-keeping gene. RESULTS: Control HL-60 cells were generally CD14(-) (18.7±4.8%) and ICOSLG(+) (93.7±1.9%). HL-60 myeloid leukemia cells showed a gradual increase in CD14 expression upon treatment with IFN-gamma while ICOSLG levels were decreased, accordingly. On the CD14(+) HL-60 cells, ICOSLG expression was more stable compared to the CD14(-) subpopulation. ICOSLG downregulation was pronounced following extended IFN-gamma exposure (>=64 hours; range, 53.2-31.5%). On the other hand, there was only a slight decline in ICOSLG expression at the transcriptional level.

DISCUSSION: Our results may indicate a possible negative regulation of ICOSLG expression in response to IFN-gamma which coincides with monocytic differentiation in myeloid leukemia. The functional consequences and mechanism of this costimulatory modulation will be further investigated.

Keywords: Myeloid leukemia, ICOSLG, costimulation, IFN-gamma

P-075 *ABSTRACT REF.: 049*

MODULATION OF CO-STIMULATORY MOLECULE EXPRESSION ON HL-60 MYELOID LEUKEMIA CELLS STIMULATED WITH ATRA, VITAMIN D3, LPS, AND IFN-GAMMA

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INTRODUCTION: All-trans retinoic acid (ATRA) and 1α,25-dihidroxyvitamin D3 (D3) which are the active metabolites of vitamin A and vitamin D, respectively, can influence maturation, differentiation, and activation of immune cells. Since they can reduce proliferation and stimulate maturation of immature acute myeloid leukemia (AML) blasts, these vitamins found application in leukemia therapy. In this study, the effect of ATRA and D3 on the expression of costimulatory molecules expressed by AML cells whose myelo/monocytic differentiation was also simultaneously induced was assessed.

METHODS: HL-60 served as the model myeloid leukemia cell line. Optimal concentration and incubation periods for ATRA and D3 were determined according to the expression kinetics of myeloid markers CD11b, CD11c, CD14, CD16, CD15 CD66b, and HLA-DR. ATRA- or D3-induced HL-60 and control cells were also stimulated with IFN-γ or LPS. Cells were stained with Giemsa for morphological analysis. Changes in the expression of surface molecules (CD86, CD80, TLR4, CD62L, CD40, B7-H1, B7-H2, B7-DC, TRAIL, and CD70) and in reactive oxygen species (ROS) production were determined by flow cytometry. Nitric oxide (NO) levels were assayed with colorimetric Griess reaction.

RESULTS: ATRA or D3 induced different maturation steps that correlated with CD11b, CD11c and CD14 expression, granulocytic or monocytic morphology and ROS production in HL-60 cells. These agents increased the cells sensitivity to LPS or IFN-γ. Correspondingly, the amount of B7-H2 positivity was decreased whereas CD86, B7-H1, and B7-DC were increased especially on which identified with high CD11b expression.

DISCUSSION: Immature myeloid leukemia blasts may change their co-stimulatory capacity in response to ATRA and D3. Especially in the patients undergoing ATRA therapy, our results may indicate a possible immune modulatory mechanism in the anti-leukemia immune responses.

Keywords: Leukemia, ATRA, D3, co-stimulation

P-076 *ABSTRACT REF.: 007*

PRIMARY PLASMA CELL LEUKEMIA: EVALUATION OF A RARE DISEASE

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The patient admitted to our hospital with complaints of anemia. General physical examination was unremarkable except for pallor. The outstanding laboratory findings were as follows: hemoglobin 8.7 g/dL, leukocyte 10.33x103/uL, platelet 56x 103/uL, blood urea nitrogen 38 mg/dL, serum creatinine 2.2 mg/dL, uric acid 14.4 mg/dL and lactate dehydrogenase (LDH) 280 u/L. He didn't have any organomegaly. Bone marrow examination showed nearly 80% of plasma cell constitution. Serum protein electrophoresis showed normal findings with a suspected monoklonal spike in gamaglobulin fraction. In immunfixation electrophoresis free kappa chain was detected. Immunohistochemical examination of bone marrow showed diffuse staining with CD20, CD38, CD117 and kappa was positive. In immunophenotypic examination of bone marrow was observed %55 CD38, %55 CD138, %55 CD19, %55 CD117 positive and kappa monoclonality in CD45 negative blast cells. However, CD56 expression was not found. Based on overall findings, the diagnosis was primary plasma cell leukemia (pPCL) in light chain.

PCL is a rare cancer involving plasma cells. In PCL, plasma cells more frequently express CD20 antigen than those of multiple myelom (MM) and often lack CD56 which is present on the majority of cells. PCL is more frequent in light chain and less frequently seen in IgA and IgG myeloma. The pPCL form occurs in patients without preceding MM or monoclonal gammopathy of undetermined significance, and the secondary form arising as a leukemic transformation of MM. As the case showed a typical presentation for pPCL and a rare, aggresive type of cancer, we wanted to report the case.

Keywords: Primary plasma cell leukemia, multiple myeloma

P-077 *ABSTRACT REF.: 054*

IL-32 AND IL-21 GENE EXPRESSION LEVELS IN PATIENTS WITH COLON CANCER

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Introduction
Immune system cells and released cytokines in tumor microenvironment define the immuno-phenotype of the tumors and take very important role on prognosis. IL-32 and IL-21 are pro-inflammatory cytokines released by immune cells. IL-32 is released by Th, NK and macrophages whereas IL-21 is released by Th, Tfh, Th17 and NKT cells. The expression levels of these cytokines vary according to the tissues where tumor is located. Present study aims to investigate the correlation between IL-32, IL-21 gene expression levels and clinico-pathological parameters in colon cancer.

Methods
31(17F) patients with diagnosis of colon cancer were included. Samples were obtained from normal and tumor tissues. After RNA isolation, IL-21 and IL-32 gene expression levels were measured using real-time PCR. The relative gene expression levels in tumor tissues were calculated using 2-ddCt method. The relations between expression levels and tumor differentiation, tumor stage, presence of vascular, neural invasions and lymph node metastasis were investigated.

Results
While IL-32 gene expression levels were found to be increased in tumor tissues (median: 1.16), IL-21 levels were found to be decreased (median:0.911). IL-32 expression levels were tended to decrease in early and advanced tumors, but were found to be increased in other stages which were also in correlation with the pathological stages of the tumor. IL-32 expression levels were

also decreased with the increased number of the lymph nodes with metastasis. There was no correlation between IL-21 expression levels and tumor differentiation, stage, presence of metastasis, vascular and neural invasion.

Conclusion
There might be a role for cytokines on tumor growth and the gene expression levels of IL-32 might give an information on the stages of colon cancer. Further studies with large number of patients are required to support our findings and to evaluate the correlations between the increased levels of IL-32 expressions and the type of the immune cells infiltrating tumor microenvironment.

Keywords: IL-32, IL-21, colon cancer, gene expression, tumor immunology

P-078 *ABSTRACT REF.: 106]*

DEVELOPMENT AND OPTIMIZATION OF AN HPLC-UV ASSAY FOR MEASUREMENT OF INDOLEAMINE2.3-DIOXYGENASE (IDO) ACTIVITY

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BACKGROUND: Indoleamine2.3-dioxygenase (IDO) is an intracellular enzyme that catalyses the initial rate-limiting step in tryptophan (Trp) degradation along the kynurenine pathway (Kyn). This tryptophane catabolic enzyme plays a crucial role in regulating the immune response by inhibiting T-cell proliferation. This immunomodulatory function of IDO plays an essential role in different physiological, paraphysiological (pregnancy), and pathological (viral infections, cancers ...) states. Measurement of IDO activity in plasma could constitute a relevant biomarker for all these different states.

OBJECTIVE: The aim of this work was to develop and optimize a rapid and specific HPLC method for measurement of IDO activity. IDO activity has conventionally been represented as a ratio of Kyn to Trp. We developed a specific HPLC method with UV detection using experimental design methodology for simultaneous measurement of Trp and Kyn in human plasma in a single run.

METHOD: The chromatographic experiments were carried out on a VARIANTM (ProStar). Plasma samples treatment consisted on deproteinization. A strategy for experimental design (factorial design and desirability function) were used to allow analyses parameters (acetonitrile percentage, buffer pH and the flow rate of the mobile phase) to be simultaneously optimized in order to identify Trp and Kyn with a high resolution between peaks, reproducibility and short analysis time.

RESULTS: Optimized analyses were run using the mobile phase consisting of 15mmol/L phosphate buffer (pH3.5) and 10.6% acetonitrile at a flow rate of 1.2ml/min. The eluate was monitored by the programmed wavelength detection setting at 360 nm from 0 to 6.5 min for Kyn and from 6.5 to 8.5 min for Trp. The linearity of the method was from 0.52 µmol/L to 21 µmol/L for Kyn and from 10 µmol/L to 400 µmol/L for Trp. Satisfactory precisions and recoveries were obtained by this method. CONCLUSIONS: Experimental design methodology allowed to rationally and economically developing a simple, fast and reliable method for measurement of IDO activity.

Keywords: indoleamine2,3dioxygenase, HPLC-UV, experimental design

P-079 *ABSTRACT REF.: 045*

SECONDARY ECHINOCOCCUS INFECTION AND CANCER METASTASIS TARGET THE LIVER UPON REDUCTION IN TYPE 1 T HELPER (TH1) IMMUNE RESPONSES

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INTRODUCTION: Immune reactions regulated by helper T cells play a decisive role during the spread of parasitic infections through internal organs. A protective effect against cancer development has been postulated for the individuals infected with E. granulosus however hydatid disease can also co-exist in cancer patients. The aim of this study was to determine the relationship between secondary E. granulosus infection and cancer metastasis in immunological assets.

METHODS: Experimental echinococcosis and breast cancer models were established with inoculation of protoscoleces and 4T1 tumor cells, respectively, into BALB/c mice. Tumor and hydatid cyst formation, and metastases were determined by macroscopic and histopathological evaluation. Distribution and characters of T cells in spleen, liver, and tumor were analyzed with flow cytometric analysis of CD3, CD4, CD8, CD25, CCR5, CCR3, IL-4, and IFN-γ markers.

RESULTS: Increased frequency of cancer metastasis and hydatid cyst formation was observed in the liver. The amount of CD4+ T cells was increased in the liver and the spleen of mice infected with E. granulosus. However, co-existence of parasitic and metastatic lesions in the liver was associated with significant reduction in the IFN-γ+ and CCR5+ Th1 and increase in the CD25+ regulatory T (Treg) cells.

CONCLUSION: Our results indicate a mutual effect of echinococcosis and cancer metastasis on immunity that allows both diseases to target and flourish in the liver.

Keywords: E. granulosus; hydatid cyst; Treg; IFN-γ; breast cancer

P-080 *ABSTRACT REF.: 074*

EXPRESSION OF TOLL LIKE RECEPTORS IN LUNG CANCER AND IINVESTIGATING THEIR EFFECTS ON APOPTOSIS

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BACKGROUND: Members of the Toll like receptors (TLRs) family play key roles in both innate and adoptive immune responses. TLRs proteins enable host to recognize a large number of pathogen associated molecular patterns such as bacterial lipopolysaccharides (LPS), viral RNA, CpG containing DNA. TLRs are also able to mediate responses against host molecules including breakdown products of tissue matrix, heat shock proteins and reactive oxigene species. Therefore, TLRs are involved in the development of many pathological conditions including cancer and infectious disease. Lung carcinoma is one of the leading causes of death worldwide. It is a non-immunogenic cancer, resistant to immune surveillance. Given that cancerous cells evade the immune system, the activation of TLRs could represent a potential

target for cancer therapy. The aim of our study, is to investigate the expression of TLRs and the effects of TLRs agonists on proliferation, apoptosis and cell cycle in SCLC-21H and NCI-H82 human non small cell lung carcinoma cell lines.

METHODS: The expression of TLR2, TLR3, TLR4, TLR5, TLR6, TLR7 SCLC-21H and NCI-H82 non small cell lung cancer cell lines were assessed by using flow cytometry. Cell cycle analysis and apoptosis were detected by flow cytometry..

RESULTS: We found high TLR2, TLR5 and TLR6 expression in SCLC-21H and NCI-H82 non small cell lung cancer cell lines. In contrast to this SCLC-21H and NCI-H82 non small cell lung cancer cell lines were showed TLR3, TLR4, TLR7 weak expression. Incubation of SCLC-21H and NCI-H82 non small cell lung cancer cell lines with TLR 2 (P. gingivalis LPS-TLR2 ligand), TLR 5 (Flagellin from B subtilis, TLR 5 ligand) and TLR6 (Diacylated lipoprotein FSL-1 TLR6 ligand) ligands induced apoptosis.

CONCLUSIONS: The expression of TLR 2, TLR5 and TLR 6 in SCLC-21H and NCI-H82 non small cell lung cancer cell lines might affect treatment approaches using TLRs agonists and shows that lung cancer cells can be regarded as active players in tumor-immunology.

Keywords: Toll like receptors (TLRs), Lung Cancer, TLRs agonists

P-081 *ABSTRACT REF.: 041*

THE CENP-F EXPRESSION PATTERN IN PATIENTS WITH COLORECTAL CARCINOMA

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Centromere protein F (CENP-F), also named mitosin, is a cell cycle-regulated protein associated with kinetochores, the site at which chromosome-microtubule interactions are monitored and the source of checkpoint signals. Its dynamic temporal expression and modification patterns, as well as its ever-changing spatial distribution during cell cycle progression, imply that it is a multifunctional protein. Growing lines of evidence have highlighted its roles in a wide variety of cellular activities, including mitotic control, microtubule dynamics, gene regulation, and muscle cell differentiation. The aim of the present study was to clarify the expression of CENP-F in human cancers.Here, we present three CENP-F-positive cases of colorectal carcinoma.

This study retrospectively analyzed the records of 5112 patients diagnosed with suspected autoimmune diseases and tested for antinuclear antibodies (ANA) between May 2012 and March 2013 at Sakarya University Teaching and Research Hospital (Sakarya, Turkey). The study included 36 patients who were diagnosed with cancer.

A total of 13 (36%) males and 23 (64%) females were studied. Antinuclear antibody positivity was detected for patient samples that were analyzed retrospectively with various titers and patterns. Indirect fluorescence microscopic studies observed five nucleolar, five nuclear granular, two cytoplasmic granular, one mitotic granular, and three CENP-F antinuclear antibody-positive patterns. Furthermore, the CENP-F-positive pattern was accompanied by a weak granular cytoplasmic pattern.

This is the first study to show CENP-F positivity in colorectal cancer patients. Our data suggest that colorectal carcinoma represents a specific biological status of cell proliferation with kinetochore proteins. Therefore, the detection of CENP-F positivity could aid in the diagnosis and monitoring of colorectal carcinoma.

In conclusion, these findings suggest an important role for CENP-F in the development and progression of colorectal and other carcinomas. In addition, CENP-F might be used to predict or identify patients who will show pathological findings. However, further study is needed to clarify the mechanism by which CENP-F is involved in the development and progression of carcinoma.

Keywords: Centromere protein F, colorectal carcinoma, cancer

P-082 *ABSTRACT REF.: 075*

COMBINED EFFECTS OF IL-8 AND CXCR2 GENE POLYMORPHISMS ON BREAST CANCER SUSCEPTIBILITY AND AGGRESSIVENESS

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BACKGROUND: Interleukin-8 (IL-8) is a prototype of the ELR+CXC chemokines that play an important role in the promotion and progression of many human cancers. We have recently showed the implication of polymorphism (-251) T/A of IL-8 gene in the susceptibility and prognosis of breast carcinoma. IL-8 acts through its CXCR2 receptor. CXCR2, expressed on the endothelial cells, is the receptor involved in mediating the angiogenic effects of ELR+CXC chemokines and in particular IL-8.

In this study, we investigated the susceptibility and prognostic implications of the genetic variation in CXCR2 in breast carcinoma. We also confirmed the implication of IL-8 (-251) T/A polymorphism in a larger cohort. Finally, we combined the IL-8 and CXCR2 variant alleles and analyzed their effects in breast cancer risk and prognosis.

METHODS: We used the allele-specific polymerase chain reaction to characterize the variation of IL-8 and CXCR2 for 409 unrelated Tunisian patients with breast carcinoma and 301 healthy control subjects. To estimate the relative risks, Odds ratios and 95% confidence intervals were calculated using unconditional logistic regression after adjusting for the known risk factors for breast cancer. Associations of the genetic marker with the rates of breast carcinoma-specific overall survival and disease-free survival were assessed.

RESULTS: A highly significant association was found between the homozygous CXCR2 (+1208) TT genotype (adjusted OR = 2.89; P = 0.008) and breast carcinoma. A significantly increased risk of breast carcinoma was associated with IL-8 (-251) A allele (adjusted OR = 1.86; P = 0.001). The presence of two higher risk genotypes (the TA and TT in IL-8, and the TT in CXCR2) significantly increased the risk of developing breast carcinoma (adjusted OR = 4.15; P = 0.0004). The CXCR2 (+1208) T allele manifested a significant association with an aggressive phenotype of breast carcinoma as defined by a large tumor size, a high histological grade, and auxiliary's lymph node metastasis. A significant association between the IL-8 (-251) A allele and the aggressive form of breast carcinoma was also found.

Moreover, the presence of the IL-8 (-251) A and/or the CXCR2 (+1208) T allele showed a significant association with a de-

creased overall survival and disease-free survival in breast carcinoma patients.

CONCLUSION: Our results indicated that the polymorphisms in IL-8 and CXCR2 genes are associated with increased breast cancer risk, as well as disease progress, supporting our hypothesis for IL-8 and ELR+CXC chemokine receptor (CXCR2) involvement in breast cancer pathogenesis.

Keywords: breast cancer, IL-8, CXCR-2

P-083 *ABSTRACT REF.: 115*

CYTOKINE EXPRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA AND CORRELATION WITH RAI CLASSIFICATION

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Chronic Lymphocytic Leukemia (CLL) is mostly originated from B lymphocytes. Rai classification system is useful to predict treatment requirements and survival rates for CLL patients. Many researchers have recently examined the cytokine content of T cells in CLL patients, and emphasized the significance of T cell activity in prognosis of the disease. IL-4 production by T cells has been shown to significantly increase in patients with progressive disease. Expression of the protein tyrosine kinase ZAP70 has been described as very valuable prognostic factor. Patients with ZAP70 positivity are characterized by worse clinical course and a significantly shorter progression-free and overall survival. In this study, intracytoplasmic IFN-gamma and IL-4 content of T, B and CLL cells in CLL patients and healthy subjects correlation with ZAP70 expression and Rai stage were investigated.

All patients diagnosed or in follow-up in Istanbul University, Istanbul Medical Faculty, Hematology Department. They had Rai staging or categorized by ZAP70 positivity. IL-4, IFN-gamma and ZAP70 expressions in peripheral blood T, B and CLL cells were measured by four color flow cytometry. ZAP70 positivity significantly correlated with Rai stage II, IV of advanced disease. Although IL-4 secreting ZAP70+ and also ZAP70- cells were significantly increased ($p<0.004$, $p<0.002$, respectively) in CD3+ T cells, IFN-gamma secretion was significantly decreased in ZAP70 and ZAP70- patients compared to healthy individuals ($p<0.006$, $p<0.002$, respectively). In contrast to T cells, IL-4 secreting CD19+ B cells showed significant decrease in ZAP70- CLL patients compared to health subjects ($p<0.004$). IFN-gamma and IL-4 secreting CD3+ T, CD19+ B and CLL cells did not show any difference in Rai stage 0-1 and Rai stage 2-4.

These findings suggest damage in the cellular immunity in ZAP70 positive CLL patients. Early cancer detection and disease stratification or classifications are critical to successful treatment. The cause of cytokine alteration in cancer immunity is not clear. Searching for the roles of cytokines in CLL is a new topic and it should be analysed further.

Keywords: Rai, ZAP70, IL-4, IFN-gamma

P-084 *ABSTRACT REF.: 122*

CD200FC SUPPRESSES LUNG METASTASIS OF 4THM BREAST CARCINOMA CELLS AND INCREASES IFN-GAMMA RESPONSE

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CD200 is a widely expressed cell surface glycoprotein that regulates excessive inflammation in autoimmunity, transplantation, and viral infections. We previously observed that viseral metastasis of highly aggressive and inflammatory 4THM breast carcinoma cells markedly decreased in CD200 transgenic mice. The goal of this study was to determine whether exogenous exposure to CD200 mimics the effects of endogenously over expressed CD200.

Female Balb-c mice were injected with CD200fc iv/ip two days after orthotopic injection of 4THM cells. In order to determine immune response to tumor cells, Mix Leukocyte cultures (MLC) were prepared from spleen and draining lymph nodes 12 and 25 days after injection of tumor cells. Extent of lung metastasis was determined macroscopically 25 days after injection. CD200fc injection significantly decreased lung metastasis without affecting the growth of primary tumors. CD200fc injection also enhanced tumor-induced IFN-g response. In addition IL-10 response to tumor cells were also suppressed in CD200fc-injected animals. These results demonstrated for the first time that CD200 analogues might have therapeutic potential in treatment of aggressive inflammatory breast carcinoma.

Keywords: CD200, Breast cancer, IFN-g

P-085 *ABSTRACT REF.: 123*

AUTOCRINE INHIBITION OF MIP-2 SECRETION FROM METASTATIC BREAST CANCER CELLS IS MEDIATED BY CXCR-2 RECEPTOR

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BACKGROUND: MIP-2, the functional homologue of IL-8 is a CXC chemokine. Effects of MIP-2 are mediated by a receptor designated as CXCR-1 and CXCR-2. Previous studies demonstrated that MIP-2 may contribute to tumor progression through regulation of angiogenesis, cancer cell growth and survival. The role of MIP-2 and its receptors in breast cancer metastasis, however is not clear. The goal of the study is to characterize MIP-2 pathway in liver and brain metastatic subset of 4T1 murine breast carcinoma cells and to determine its significance on metastatic growth.

MATERIALS-METHODS: 4THM cells were originally derived from myocardial metastasis of 4T1 cells. 4TLM (4T Liver Metastasis) and 4TBM (4T Brain Metastasis) cells were from liver and brain metastasis of 4THM cells respectively. MIP-2 levels in the conditioned medium were measured from each cell line at different time points. CXCR2 and CXCR1 expression was measured using flow cytometry. CXCR2 antagonist SB225002 was used to evaluate the role of CXCR2 on cell proliferation and MIP-2 secretion.

RESULTS: Both brain and liver metastatic cells secreted significantly higher amounts of MIP-2 levels compared to parental 4T1 cells. These cells predominantly expressed CXCR2. Inhibition of CXCR2 activity with SB225002 dose-dependently decreased

cell proliferation. Unexpectedly CXCR2 blockage significantly increased MIP-2 secretion.

CONCLUSIONS: These results demonstrated for the first time that MIP-2 and CXCR2 receptors are involved in growth of brain and liver metastatic cells of breast carcinoma and CXCR-2 antagonist might have therapeutic potential in metastatic breast cancer.

Keywords: MIP-2, Breast cancer, CXCR2, chemokines

P-086 *[ABSTRACT REF.: 014*

CCRL2 ATYPICAL CHEMOKINE RECEPTOR VARIANTS' EXPRESSION AND FUNCTIONAL ANALYSIS IN BREAST CANCER CELLS

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Atypical chemokine receptors play role in the termination of inflammatory responses and CCRL2 is the newest member. CCRL2 binds CCL5, CCL19 and chemerin and decreases their local concentration. Therefore, immune cell migration is hampered. Human CCRL2 gene has two variants; namely, CRAM-A and CRAM-B. The aim of this work is to investigate the expression and the functions of these variants in breast cancer cells.

CRAM-A and CRAM-B expression were determined with RT-PCR in breast cancer cell lines, PBMCs, and purified-immune cells under IFN-γ or LPS stimulation. pCRAM-A-IRES2-EGFP and pCRAM-B-IRES2-EGFP recombinant DNAs were constructed and confirmed by PCR, restriction-digestion and DNA-sequencing. These recombinant-plasmids and the empty-vector were transfected into HEK293T cell line and MDA-MB-468 and BT-474 breast cancer cell lines. Transfection efficiency (GFP-expression) and recombinant CRAM expression were examined by flow cytometry. For functional analyses; Ca2+ flux (FuraRed II staining), ligand-binding, receptor-internalization and ligand-removal assays were performed with or without CCRL2-blocking antibodies. Recombinant CCRL2 constructs were successfully expressed in HEK293T cells (GFP+ CCRL2+ 66.3-84.22%). As expected, CCL5, CCL19 and chemerin did not stimulate intracellular Ca2+ flux, whereas ionomycin Ca2+ ionophore did. On breast cancer cells, CRAM-A expression was specifically increased upon IFN-γ stimulation. In the presence of chemokine ligands, CRAM-A internalization was determined in ~30 minute-intervals. In addition, CCL19 was the most efficiently removed chemokine from the environment. This effect was observed both in HEK293T and BT-474 cell lines transfected with recombinant CCRL2. Therefore, CRAM-A expression may serve as an immune evasion mechanism that mitigates T cell infiltration towards the tumor.

Keywords: Atypical chemokine receptor, CCRL2, Chemokine, Recombinant DNA technology.

P-087 *ABSTRACT REF.: 080*

T HELPER RESPONSES ARE MAINTAINED BY BASAL-LIKE BREAST CANCER CELLS AND CONFER TO IMMUNE MODULATION VIA UPREGULATION OF PD-1 LIGANDS

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INTRODUCTION: Even though a high cytotoxic T cell infiltration is generally associated with favorable prognosis, the immunological course of breast cancer is explicitly directed by helper T cells. This study aims to determine the influence of BLBCs on CD4+ T cell responses.

METHODS: Co-cultures were established between breast cancer cell lines and CD4+ T cells under stimulatory conditions. Helper T cell activation, proliferation, cytokine secretion, and differentiation were assessed. Protein and mRNA expression of PD-1 ligands were determined on breast cancer cell lines. Blockage assays were performed in order to determine the functional assets of PD-1 ligation.

RESULTS: In contrast to luminal breast cancer cells, BLBC cells allowed CD4+ T cell activation, proliferation, and IFN-γ secretion, but only to a certain extent. In return, IFN-γ stimulated the upregulation of PD-L1 and/or PD-L2 on the basal-like cells. A substantial population of CD25+CD127low/- regulatory T (Treg) cells was also induced in BLBC co-cultures. Accordingly, in prolonged periods of co-culturing, blockage of PD-1 ligands on BLBC cell lines impaired Treg differentiation, restored IL-2 secretion, and increased CD8+ T cell activation. CONCLUSIONS: T helper responses were permitted by BLBC cells. On the other hand, IFN-γ secreted from Th1 and other immune cells upregulated the expression of PD-1 ligands on BLBC cells and modulated the immune reactions. Our results indicate the capacity of BLBCs to adapt to IFN-γ-mediated immune responses and to evade immunity via upregulation of PD-1 ligands.

Keywords: Basal-like breast cancer; T lymphocyte; immune modulation; PD-1.

P-088 *ABSTRACT REF.: 077*

TYROSINE 416 IS PHOSPHORYLATED IN THE CLOSED, REPRESSED CONFORMATION OF C-SRC

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c-Src signaling controls many cellular events such as cell growth, proliferation, differentiation, motility and cell adhesion. The kinase activity of c-Src depends on whether the protein is in the more expanded "open" active conformation or in the more compact "closed" repressed conformation. Phosphorylation of Y527 facilitates the formation of the closed conformation by enabling high affinity binding of the SH2 domain to the C-tail. This interaction, as well as binding between the SH3 domain and the SH2-kinase linker, creates a compact structure that represses kinase activity. Dephosphorylation of Y527 releases SH2 binding to the C-tail leading to a more open conformation with far greater kinase activity. Here we investigated the correlation of Y416 phosphorylation with c-Src activity when c-Src was locked into the open and closed conformations (by mutations Y527F and Q528E, P529E, G530I respectively). Consistent with prior findings, we found Y416 to be more greatly phosphorylated when c-Src was in an open, active conformation. However, we also observed an appreciable amount of Y416 was phosphorylated when c-Src was in a closed, repressed conformation under conditions by which c-Src was unable to phosphorylate substrate STAT3. The phosphorylation of Y416 in the closed conformation arose by autophosphorylation, since abolishing kinase activity by mutating the ATP binding site (K295M) prevented phosphorylation. Basal Y416 phosphorylation correlated positively with cellular levels of c-Src suggesting autophosphorylation depended on self association. Using sedimentation velocity analysis on cell lysate with fluorescence detection optics, we confirmed that c-Src forms monomers and dimers, with the open conformation also forming a minor population of larger mass complexes. Collectively, our studies suggest a model by which dimerization of c-Src primes

c-Src via Y416 phosphorylation to enable rapid potentiation of activity when c-Src adopts an open conformation. Once in the open conformation, c-Src can amplify the response by recruiting and phosphorylating substrates such as STAT3 and increasing the extent of autophosphorylation.

Keywords: c-Src, Tyrosine 416, autophosphorylation

P-089 *ABSTRACT REF.: 098*

INDUCTION OF IL-33 IN GLIOBLASTOMA MULTIFORME TISSUES

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Interleukin-33 (IL-33), discovered at 2005, is a novel multifunctional IL-1 family cytokine. It mediates its biological effects via interaction with the receptors ST2 (IL-1RL1) and IL-1 receptor accessory protein (IL-1RAcP), both of which are widely expressed, particularly by innate immune cells and T helper 2 (Th2) cells.

The functions of IL-33 and its producing cells in the central nervous system (CNS) are still uncertain. The purpose of this study was to determine the correlations between protein IL-33 levels and the pathogenesis in GBM patients. In this study, we investigated the protein level of IL-33 in the GBM. The expression of IL-33 protein was upregulated in GBM tissues in comparison with matched normal tissues. As a result of 3 fold IL-33 increases implicating that this new cytokine might be involving in GBM pathogenesis.

Therefore, IL-33 may be considered as an important mediator in the regulation of Glioblastoma Multiforme cancer progression.

Keywords: IL-33, Glioblastoma Multiforme,Western Blotting

P-090 *ABSTRACT REF.: 000*

CHARACTERIZATION OF HLA-G EXPRESSING HUMAN NK CELLS

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Background. Natural killer (NK) cells have been long time considered as cytotoxic cells integrated into effector branch of innate immunity during antiviral, antitumoral, antimicrobial, and antiparasitic response. However, it is noted that NK cells also play positive and negative regulatory effect to innate and adaptive immunity by secreting various cytokines or cell-to-cell contact and maintain immune homeostasis. HLA-G is an important immune-regulatory molecule, which is restricted to a very limited number of normal tissues. The ability of HLA-G to inhibit NK cell-mediated lysis has been recently shown. Ligation of NK cell receptors (KIR, NKG2A and ILT2) responsible for HLA-G recognition resulting in the inactivation of NK cell effector function. Moreover, HLA-G expressing non-cytolitic NK regulatory cells have been generated in vitro from human hematopoetic progenitors. Their immune regulatory effects were represented by the surface expression/release of HLA-G. In this study, cytokine profile and immunosuppressive activity of HLA-G+ NK cells were investigated.

Methods. Study group consists of healthy subjects (n=7, mean age=34.3 ± 9.8). HLA-G expression were determined in freshly purified NK cells and after their incubation in presence of PHA (10 µg/ml) and IL-2 (20 ng/ml) alone or in combination during 18 hours by flow cytometry. TGF-β, IFN--10 secretion was assessed after 18 hours of incubation of NK cells with PHA and Brefeldin A. To investigate the cytotoxic activity of HLA-G- NK cells, sorted NK cells were activated overnight with PHA, and then HLA-G+ and HLA-G- NK cells were isolated. In presence of K562 (5:1), cytotoxic activity, CD107a expression and perforin content were analyzed.

Results. Freshly purified NK cells contain a small subset, which constitutively express HLA-G and represent about 5% of the total NK cells. HLA-G expression is significantly increased in presence of PHA compared to unstimulated NK cells (p=0.0002), while the effect of IL-2 alone or in combination with PHA were slightly lower (p=0.19 and p=0.09, respectively). According to the cytokine secretion, IL-10 (68%) and TGF-β (80%) producing HLA-G+ NK cells were higher compare to HLAG- NK cells. Addition of HLA-G+ NK cells into the HLA-G- NK cell culture were significantly decreased cytotoxicity but did not effect on expression of perforin, CD107a and IFN-expression (p=0.08).

Conclusion. Taking together, human peripheral blood NK cells contain a subset, which express suppressive molecule - HLA-G and secrete two major immunoregulatory cytokines, which can down-regulate NK cell cytotoxic activity. HLA-G+ NK cells represent as a novel suppressive subset of NK cells could be employed to new immunosuppressive strategies.

P-091 *ABSTRACT REF.: 000*

GENETIC POLYMORPHISM OF IL23R ALTERS RECEPTOR EXPRESSION LEVELS IN CD4 T CELLS

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Objective.
IL23R gene polymorphisms have been identified as risk factors for major inflammatory bowel diseases and also with Behcet's disease (BD). The association of a variant of IL23R gene (rs17375018) with BD has been reported in Chinese population and replicated in Turkey. However how this polymorphism contributes to disease is poorly understood. IL23R is enriched on Th17 cells and IL-23 plays a role in the sustenance of Th17 cells in vivo. In this study, we investigated the expression levels of the IL23R in donors carrying different genotypes with possible functional activities related to polymorphism.

Methods.
CD4+ T cell population was isolated from 26 healthy donors genotyped for IL23R polymorphism (rs17375018). IL-23R and IL17A mRNA levels were determined semi-quantitatively by PCR using Sybr Green. Quantitative evaluations were performed by comparing GAPDH.

Results.
Expression of IL-23R was lower in rs17375018 AA carriers than in GG (p=0.014) and AG (p=0.018). The presence of A allele (AA

+ AG) also decreased the expression level of the receptor in CD4+ compared to donors without A allele of IL23R variant (p= 0,0001). The expression levels of IL17A in ex vivo CD4+ T cells were not different related to the genotypes.

Conclusion.
Alterations of the IL23R expression in CD4 T cells may have implications in the disease pathogenesis and contribute to the IL-17 induction ın diseases associated with this gene polymorphism.

P-092 *ABSTRACT REF.: 000*

REGULATION OF A20 GENE EXPRESSION BY MIRNA IN ENDOTHELIAL CELLS EXPOSED TO HIGH CONCENTRATION OF GLUCOSE

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It is known that apoptosis is induced in *in vitro* endothelial cells that were exposed to glucose levels similar to that found in blood of diabetic patients. A20 gene was described as an anti-apoptotic endothelial protective gene. In this study, we investigated if microRNAs could modulate gene expression of the A20 gene in endothelial cell when the cells were exposed to high concentration of glucose.

Methods: The sEND-1 endothelial cells were cultured in 33mM (high) glucose containing media in the presence and absence of insulin for different time points. Activation of cells was monitored by IL6 secretion (ELISA) and IkB-alpha expression (RT-PCR). A20 expression of cells was measured by RT-PCR, Western Blotting, and fluorescent microscopy. Finding which if any miRNA regulates the A20 gene expression, 3'UTR region of A20 was amplified and fused to the *Renilla* luciferase open reading frame of the dual luciferase reporter vector, HEK293 cells were co-transfected with them and with the expression vectors encoding the *in silico* predicted miRNAs, and luminescence measurement for each miRNA was recorded when incubation was completed in culture. SNP at positions 1555 (T→G), 1561 (T→C), and 1676 (T→C) were detected within the 3'UTR region of the cell genome, and was corrected on insert by molecular engineering before its cloning to the pCR2.1 amplification vector. Specific miRNA expression in activated endothelial cells was measured by TaqMan PCR.

Results: miR-424 out of 24 miRNAs, which were predicted targeting the A20 3'UTR, significantly repressed Renilla expression in dual reporting system suggesting miR-424 was repressing the A20 expression. In high glucose concentration, the endothelial cells became activated, increased mRNA expression of A20 and miR-424 genes, and increased A20 protein expression with the highest level at day two. The results support the conclusion that A20 gene expression in endothelial cells under high glucose concentration is regulated by miR-424, suggesting targeting miR-424 by antagomirs could be a feasible pharmaceutical approach to increase the expression of protective A20 within dysfunctional endothelial cells.

P-093 *ABSTRACT REF.: 000*

EVIDENCE FOR GENETIC REVERSION IN LEAKY SCID MICE

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Objective: We wanted to understand whether scid T cell leakiness might in part reflect secondary mutations in DNA-PK catalytic subunit gene locus which might allow some rare cells to make successful V(D)J gene rearrangements to give functional B or T lymphocytes.

Method: Lymphocyte enriched cells were isolated from mouse spleen to use as feeder cell after irradiation. Thymic T cell lines 1287, 1374, 7176, 1280 and 1233 from leaky scid mice were cultured on the feeder cells. Total DNA was isolated from the T cells, and also from the control cells 1142 (scid B), AC11 (wild type B), 7583, and SN1. To test the for radio sensitivity of the cells, they were irradiated between 25-300 cGY for 17- 120 sec and than cultured for 24-96 h before viability counting. Multiple primers were designed to amplify the region between positions 11410 and 12137 at DNA-PKcs gene locus. PCR products were tested by Alu-I digestion to screen the putative site of the scid mutation at exon 85. The PCR products were T/A cloned into plasmid pBR233, amplified, clones with insert were blue/white selected, the inserts were cut from the plasmid to be sequenced.

Results: In scid mice and all scid lines, the TAT codon in amino acid position 4045 showed a T to A point mutation at the third nucleotide, which created a TAA stop codon. In one allele of the 7167 leaky T cell line, a T to C reversion in the first nucleotide of the TAA stop codon was detected. This CAA triplet codes for glutamine, which would allow for read-through, and permit 7167 cells to make a full-length DNA-PKcs protein. The other scid clones examined did show no evidence of novel transcripts resulting from variant splicing in the region between exons 77-86.

Conclusion: This finding supports the conclusion that the scid nonsense mutation might be overwhelmed in some rare leaky cells by a genetic reversion mechanism and allows the cell to express full-length DNA-PKcs protein. The basis for leaky phenotype in other clones is still unclear.

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