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3rd International Molecular Immunology & Immunogenetics Congress (MIMIC-III)

April 27 - 30, 2016 Papillon Ayscha Hotel - Belek / Antalya - TURKEY

Wednesday, April 27th 2016		
11:00 - 16:00	REGISTRATION	
16:00 - 18:00	OPENING CEREMONY	
16:00 - 16:30	Opening Speech	Barbaros ORAL, Gunnur DENIZ
16:30 - 17:00	Interpersonal Communication	Cemal CINGI (TUR)
17:00 - 18:00	OPENING CONFERENCE: Plenary Lecture	Chairs: Barbaros ORAL Ihsan GURSEL
17:00 - 18:00	Inducing and Breaking of Allergen Specific Tolerance	Mubeccel AKDIS (SUI/TUR)
18:00 - 18:30	COFFEE BREAK	
18:30 - 20:30	WELCOME RECEPTION AND COCKTAIL	

Thursday, April 28th 2016		
08:30 - 10:30	NOVEL DISCOVERIES IN THE INNATE IMMUNE SYSTEM (Remembering PROF. OLCAY YEGIN)	Chairs: Mayda GURSEL
08:30 - 09:00	Novel Functions of Neutrophils in Inflammation and Immunity	Attila MOCSAI (HUN)
09:00 - 09:30	IL-10-Producing NKT10 cells are a Novel Regulatory iNKT Cell Subset	Gerhard WINGENDER (TUR)
09:30 - 09:45	Modulating the Differentiation of Human Monocytes into Immunosuppressive Macrophages via TLR1/2 Signaling	Defne BAYIK (USA)
09:45 - 10:00	Molecular Mechanisms for Adenosine Regulation of Dendritic Cells	Merve KAYHAN (TUR)
10:00 - 10:15	APPL1 Involvement in TLR3/4-dependent TBK1 and IKKε Activation in Macrophages	Tieu Lan CHAU (BEL/TUR)
10:15- 10:45	COFFEE BREAK	

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EFIS

European Journal of Immunology

Thursday, April 28th 2016		
10:45 - 12:15	IMPACT OF INFLAMMATION IN DISEASES	Chairs: Sefik Sanal ALKAN, Vedat BULUT
10:45 - 11:15	Purinergic Regulation of Immune Responses: Implications for Cancer Immunotherapy	Duygu SAG (TUR)
11:15 - 11:45	Cancer-Related Inflammation	Sven BRANDAU (GER)
11:45 - 12:00	The Effect of Fingolimod Treatment on Peripheral Blood Cell Immunophenotypes in Patients with Multiple Sclerosis	Canan ULUSOY (TUR)
12:00 - 12:15	A New Humanized Mouse Model for Autoimmune Cardiomyopathy and Its Use to Devise Immunomodulation Therapy	M. Emrah SELLİ (TUR)
12:15 - 13:30	LUNCH AND BREAK	
13:30 - 15:00	EMERGING ISSUES IN ADAPTIVE IMMUNE SYSTEM (Remembering PROF. OZDEMIR ILTER)	Chairs: Gulderen YANIKKAYA DEMIREL
13:30 - 14:00	Insights into Lymphoid Organs Throughout Vertebrate Evolution	Luigi ABELLI (ITA)
14:00 - 14:30	Targeting the p53 Tumor Suppressor	Batu ERMAN (TUR)
14:30 - 14:45	Effects of Gender Differences and Sex Hormones on Mouse Bone Marrow Cell Populations	Gokhan TERZIOGLU (TUR)
14:45 - 15:00	Accumulation of AID in Immunoglobulin Variable Genes in the Absence of Topisomerase I	Huseyin SARIBASAK (TUR)
15:00 - 15:30	COFFEE BREAK	
15:30 - 17:00	REGULATORY CELLS OF IMMUNE SYSTEM	Chairs: Ayca SAYI YAZGAN
15:30 - 16:00	Subsets of Human Natural Killer Cells and Their Regulatory Effect	Gunnur DENIZ (TUR)
16:00 - 16:30	IL-10 Producing Regulatory B Cells in Helicobacter Infection	Ayca Sayı YAZGAN (TUR)
16:30 - 16:45	The Effect of Activated Allogeneic Macrophages on Tumor Immunity in Experimental Lung Cancer	Gurcan TUNALI (TUR)
16:45 - 17:00	S1P1 Deletion Differentially Affects TH17 and Regulatory T Cells	Ahmet EKEN (TUR)

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Thursday, April 28th 2016	
18:30 - 20:30	TSI GENERAL ASSEMBLY MEETING
20.30 - 23:00	POSTER SESSIONS 1 - 10
20.30 - 23:00	Poster Session 1: Novel Discoveries in the Innate Immune System
	Poster Session 2: Impact of Inflammation in Diseases
	Poster Session 3: Emerging Issues in Adaptive Immune System
	Poster Session 4: Regulatory Cells of Immune System
	Poster Session 5: Tumor Immunology & Molecular Markers
	Poster Session 6: Tumor Immunotherapy
	Poster Session 7: Immunodeficiencies: Emerging Molecular & Cellular Therapies
	Poster Session 8: Immunity to Infection: New Trends and Developments
	Poster Session 9: Transplantation Immunology
	Poster Session 10: Molecular Targets in Rheumatoid Arthritis and Autoimmune / Autoinflammatory Diseases

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European Journal of Immunology

Friday, 29 April 2016		
08:00 - 10:00	TUMOR IMMUNOLOGY & MOLECULAR MARKERS	Chairs: Dicle GUC, Emin KANSU
08:00 - 08:30	The Role of Human TAMS and Monocytes in Breast Cancer	Luca CASETTA (UK)
08:30 - 09:00	KBKE Maintains a Pro-inflammatory Loop for Intestinal Tumor Development	Serkan GOKTUNA (TUR)
09:00 - 09:30	Chronic Inflammatory Microenvironment Defines the Pro-Tumorigenic Functions of IFN-gamma	Suhendan EKMEKCI-OGLU (USA/TUR)
09:30 - 09:45	ATP-binding Cassette Transporter G1 (ABCG1) is a Novel Mediator of Tumor Immunity	Duygu SAG (TUR)
09:45 - 10:00	Cancer Associated Fibroblasts Display Phenotypic and Functional Features that Resemble Circulating Fibrocytes which Constitute a Novel Subset of MDSCs	Gurcan GUNAYDIN (TUR)
10:00 - 10:30	COFFEE BREAK	
10:30 - 12:15	TUMOR IMMUNOTHERAPY	Chairs: Akif TURNA, Gunes ESENDAGLI
10:30 - 11:00	NK Cell-based Approaches in Cancer Immuno-therapy	Tolga SUTLU (TUR)
11:00 - 11:30	CpG ODN Loaded Exosomes Mediate Eradication of Established Tumors	Ihsan GURSEL (TUR)
11:30 - 11:45	Encapsulation of TLR9 and iNKT Cell Ligands into Exosomes Induces Immune Responses and Prevents Tumor Development	Gozde GUCLULER (TUR)
11:45 - 12:00	Uroplakin 3A Derived Peptide Vaccination Reduced Tumor Growth in Bladder Cancer Mice Modell	Kenan IZGI (TUR)
12:00 - 12:15	Liposomal D-and K-type CpG ODN Combination Confers Synergistic Immune Activation and Act as an Efficient Tumor Vaccine	Begum Han HORU-LUOGLU (TUR)
12:15- 13:30	LUNCH AND BREAK	

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April 27 - 30, 2016 Papillon Ayscha Hotel - Belek / Antalya - TURKEY

Friday, 29th April 2016		
13:30 - 15:30	IMMUNODEFICIENCIES: EMERGING MOLECULAR & CELLULAR THERAPIES (Remembering PROF. ISIL BARLAN)	Chairs: Yildiz CAMCIOGLU, Gunnur DENIZ
13:30 - 14:00	DOCK8 Deficiency: Cellular and Molecular Mechanisms	Talal CHATILA (USA)
14.00 - 14.30	ARTEMIS Mutations Causing Phenotypes Ranging from Atypical SCID to Mere Antibody Deficiency	Ismail REISLI (TUR)
14.30 - 15.00	Interferonopathies: A New Paradigm Shift in Primary Immune Deficiency Syndromes	Mayda GURSEL (TUR)
15:00 - 15:15	Immunodeficiency and Immune Dysregulation in DOCK8 Deficiency: Clinical and Mechanistic Studies in a Defined Patient Cohort	Sevgi KELES (TUR)
15:15 - 15:30	Personalized Therapy for X-linked Agammaglob-ulinemia (XLA) by Oligonucleotide-induced Splice-correction	Burcu BESTAS (TUR)
15:30 - 16:00	COFFEE BREAK	
16:00 - 16:45	IMMUNOLOGY DAY SPECIAL LECTURE	
16:00 - 16:45	Regulation of Chronicity in Diseases by Immune System Interaction with Resident Tissue Cells	Cezmi AKDIS (SUI/ TUR)
16:45 - 17:00	CONGRESS PHOTO SHOOTING	
17:00 - 19:00	IMMUNITY TO INFECTION: NEW TRENDS AND DEVELOPMENTS	Chairs: Tunc AKKOC, Esin AKTAS
17:00 - 17:30	Inducible Adjuvanticity During Infection and Vaccination	Ken ISHII (JPN)
17:30 - 18:00	Tissue-Specific Immunopathology During Malaria	Cevayir COBAN (JPN/TR)
18:00 - 18:30	Tiny RNAs Control Adaptive Humoral Immune Responses	Hans-Martin JÄCK (GER)
18:30 - 18:45	Phenotypic Heterogeneity in Patients with the Same IL12RB1 Mutation: Screening of 3 Families with Patients with BCG Infection	Cagman TAN (TUR)
21:30 - 23:30	WORLD IMMUNOLOGY DAY PARTY	

Saturday, 30th April 2016		
08:00 - 08:30	Rational Drug Use	Ihsan GURSEL (TUR)
08:30 - 10:30	TRANSPLANTATION IMMUNOLOGY	Chairs: Huseyin TUTKAK, Emel DEMIRALP EKSIOGLU
08:30 - 09:00	The Challenge of Transplant Rejection: From Understanding Allorecognition to Inducing Tolerance	Robert LECHLER (UK)
09:00 - 09:30	Clinical Grade Manufacture of Regulatory T Cells to Promote Transplantation Tolerance: Challenges and Achievements	Giovanna LOMBARDI (UK)
09:30 - 10:00	Cellular Therapy in Transplantation - A Dream of Good Doctor	Piotr TRZONKOWSKI (POL)
10:00 - 10:15	Donor-Recipient Killer Immunoglobulin Like Receptor (KIR) Gene-Gene Matching Improves Post-Transplant Outcomes in HLA Identical Sibling Hematopoietic Cell Transplantation: Matching Reduces Frequency of Relapse and Graft Versus Host Disease	Ugur SAHIN (TUR)
10:15 - 10:30	The Immune Escape in Ovarian Cancer: Role of Epigenetic Mechanisms in Suppression of Positive Costimulatory Molecules in Chemoresistant Ovarian Cancer Cells	Ercan CACAN (TUR)
10:30 - 11:00	COFFEE BREAK	
11:00 - 13:00	CELLULAR AND MOLECULAR TARGETS IN AUTO-IMMUNE/AUTOINFLAMMATORY DISEASES	Chairs: Guher Saruhan DIRESKENELI
11:00 - 11:30	Therapeutic Approaches for Inhibiting Intracellular Signalling Pathways in Rheumatoid Arthritis and Other Immune-Mediated Disorders	Haner DIRESKENELI (TUR)
11:30 - 12:00	Intra-Cellular Signalling Pathways in Inflammatory Disorders	Ahmet GUL (TUR)
12:00 - 12:15	Src Family Kinases Dependent STAT3 Activation is Involved in LPS-induced Cytokines Production in PBMC of Patients with Behçet's Disease	Sevgi IRTEGUN (TUR)
12:15 - 12:30	Characterization of NK Cells in Patients with Multiple Sclerosis Under Sphingosine-1-phosphate Receptor 1 (S1P1) Antagonist Therapy	Didem OZKAZANC (TUR)
12:30 - 13:00	CLOSING CEREMONY	

3rd INTERNATIONAL MOLECULAR IMMUNOLOGY & IMMUNOGENETICS CONGRESS MIMIC-III

APRIL 27-30, 2016 BELEK, ANTALYA, TURKEY

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Novel functions of neutrophils in inflammation and immunity

Attila Mócsai

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Neutrophils are double-edged swords playing a critical role in innate immune defense but also contributing to tissue damage during excessive inflammation.[1] Their exact role in those processes and the molecular details of their function are still poorly understood. We and others have recently shown that neutrophils are critically involved in various *in vivo* models of innate immunity and inflammation in experimental mice.[2] Surprisingly, neutrophils were not only found to contribute to non-adaptive immunological processes but also participated in shaping the adaptive immune response, e. g. during the sensitization phase of contact hypersensitivity.[2] Neutrophils are also able to release a number of pro-inflammatory mediators including chemokines, cytokines and lipid mediators such as LTB4. The analysis of various neutrophil-mediated inflammatory processes revealed several novel aspects of neutrophil function. Many of the mediators released by neutrophils promote the recruitment or activation of other neutrophils, therefore establishing positive feedback amplification loops during neutrophil-mediated inflammation.[3] Immunoreceptor-induced neutrophil responses required receptor-proximal Src-family kinases, Syk and PLCγ2 which were also critical for neutrophil-mediated *in vivo* inflammatory reactions.[4-6] Interestingly, the downstream gene expression regulator CARD9 was required for the release of chemokines and cytokines but not for LTB4 release or other classical neutrophil responses, providing a unique opportunity to test the *in vivo* role of neutrophil gene expression changes and neutrophil-derived chemokines/cytokines.[7] Neutrophil-specific deletion of CARD9 attenuated various different *in vivo* inflammation models,[7] providing the first direct evidence for the role of neutrophil gene expression changes during an *in vivo* inflammatory reaction.

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IL-10 producing regulatory B cells in helicobacter infection

Ayca Sayi Yazgan

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B cells have been associated in recent years with regulation of excessive inflammation.[1] The first evidence for a regulatory role of B cells was described in mouse models of experimental autoimmune encephalomyelitis.[2] After this, several studies show that B cells could suppress excessive inflammation in models of arthritis,[3] type 1 diabetes,[4] contact hypersensitivity,[5] Salmonella infection[6] and colitis.[5] Primarily, regulatory B (Breg) cells function through their IL-10, IL-35 or TGF-beta and the activation of regulatory T (Treg) cells via direct interaction both in mice and humans.[7] In mice, multiple subsets of IL-10-producing Breg cells including transitional 2 marginal-zone precursor (T_2-MZP) cells, CD5+CD1dhi B (B10) cells, marginal-zone (MZ) B cells, Tim-1+ B cells, CD138+ plasma cells, and plasmablasts.[7]

Our previous work has shown that B cells have the ability to negatively regulate adaptive immune responses to a bacterial pathogen, *Helicobacter felis (H. felis)*. Using mouse models of infection with *H. 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 both *in vitro* and *in vivo*.[8] 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*. Our recent data suggest that, *H. felis* stimulated IL-10 producing B cells exhibit a heteregenous population showing mainly CD1dhiCD5+ regulatory B10 cell phenotype that shares phenotypes with T2-MZ B cells. In addition to that, stimulation of B cells with *H. felis* leads to both IgM and IgG2b secretion from IL-10 negative B cells, but not from IL-10 positive Breg cells.

It was previously reported that, stimulation of immune cells with TLR- agonists leads to activation of several signaling pathways including the MAP kinase (p38 MAPK, ERK 1/2, and JNK) pathway, the PI3- kinase pathway, and the NF-κB pathway.[9] However the potential mechanisms explaining previously shown TLR-2- induced IL-10 production in the *H. felis* -stimulated B cells have not been analyzed. Therefore, we investigated the effect of *H. felis*- stimulation on the activation of the MAP kinase-pathway, the PI3- kinase pathway, and the NF-κB pathway in primary B cells. Our data suggest that the ERK- 1/2 rather than p38 MAPK and PI3K, are activated in *H. felis* -stimulated IL-10+ B cells. Besides, the activation of ERK1/2 is responsible for the TLR2-induced IL-10 production from B cells.

We will discuss our recent results in this talk.

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IL-10-producing NKT10 cells are a novel regulatory iNKT cell subset

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Invariant Natural Killer T (iNKT) cells are a unique subset of T cells that combine qualities of innate NK cells and adaptive memory T cells. Two main features characterize them. First, they express an invariant TCRα rearrangement, comprised of a Vα14-Jα18 TCR rearrangement (Vα14i) in mice and an orthologous Vα24-Jα18 TCR chain (Vα24i) in humans. Second, they recognize glycolipid antigens presented by CD1d, a non-polymorphic homolog of the MHC class I antigen-presenting molecules. This recognition of glycolipids distinguishes iNKT cells from conventional T lymphocytes, which recognize peptide antigens. There is a surprising degree of interspecies cross-reactivity, with mouse Vα14i NKT cells recognizing human CD1d and vice versa. This conservation over 50 million years of evolution suggests an important function for these cells in the mammalian immune system. Following TCR stimulation, for example with the model antigen α-galactosylceramide (αGalCer), iNKT cells rapidly produce copious amounts of various cytokines, including Th1 cytokines, like IFNγ and TNF, and Th2 cytokines, like IL-4 and IL-13, and Th17 cytokines. Due to this cytokine production, iNKT cells can have a pronounced effect on the immune system, impacting a dazzling variety of different immune reactions, ranging from responses against pathogens and tumors to autoimmunity. A reasonable explanation for these wide-ranging activities could be the recent insight that iNKT cells are comprised of several distinct subsets. Most prominent are NKT1, NKT2 and NKT17 cells that were named following the nomenclature of Th1, Th2 and Th17 cells due to the similarities in their cytokine production. Furthermore, we described a novel iNKT cell subset with IL-10-dependent regulatory function, which we termed NKT10 cells. NKT10 cells were detected in mice and in healthy human donors. Additionally, NKT10 cells could be expanded greatly in mice by *in vivo* stimulation with particular antigens. These were iNKT cell antigens known to bias the immune response towards Th1. Importantly, through the production of IL-10, NKT10 cells could protect mice during experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis. These data demonstrate that NKT10 cells can protect mice against a debilitating neuroinflammatory disease when they are present in sufficient numbers at the onset of the disease. Therefore, NKT10 cells represent the first iNKT cell subset with regulatory function found under resting, steady-state conditions in mice and humans. Here the characteristics, functions and the therapeutic potential of NKT10 cells will be outlined.

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Clinical grade manufacture of regulatory T cells to promote transplantation tolerance: challenges and achievements

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Long-term survival of organ transplants remains limited by chronic rejection and side effects of immunosuppression (IS). However, IS weaning post-transplantation has been largely unsuccessful, supporting the need for active tolerance induction strategies. CD4+CD25+FOXP3+ (Tregs) play an important role in immunoregulation and have been shown in animal models to promote transplantation tolerance. Phase I trials in bone marrow transplantation and Type 1 diabetes have shown that ex vivo expanded Tregs have an excellent safety profile, encouraging the broader application of these cells.

We have devised a GMP compatible protocol which ensures the successful isolation and expansion of a functional and stable human Treg lines derived from Tregs isolated from the blood of healthy controls. Four years ago this protocol was moved to the GMP facility at Guy's Hospital in preparation for two clinical trials; the One Study and ThRIL. We have demonstrated that Treg lines can be generated by stimulating Tregs obtained from the blood of end-stage kidney and liver disease patients on a waiting list for either kidney or liver transplants. Our results demonstrate that Tregs isolated from the blood of these patients using a CliniMACS-based GMP isolation technique and polyclonally expanded in the presence of IL-2 and rapamycin, were pure (97.4% CD4+CD25+) and maintained FoxP3 expression (99.6%). In addition, the Treg lines obtained were highly suppressive and stable. However, we have encountered some difficulties such as some patients not reaching the number of Tregs needed for their infusion. The One Study has been just completed and 12 patients have been injected with Tregs, while in the ThRIL the first three patients have been treated (cohort one). We are now analyzing the samples that have been collected from the beginning of the clinical trials with the aim to observe some sign of an effect of Treg treatment.

In conclusion the rapamycin-based protocol established in the laboratory is ideal in this setting as it not only satisfies the rigors of GMP manufacturing standards, but also generates a population of Tregs which is phenotypically and functionally stable and superior to freshly isolated cells. We are now in a position to cell sort the Tregs in the GMP facility at Guy's Hospital and this new approach will provide the opportunity to generate graft-specific Tregs for future cell therapies in the search for transplantation tolerance. The possibility of combining Treg therapy with other strategies will be discussed.

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DCLRE1C (ARTEMIS) mutations causing phenotypes ranging from atypical severe combined immunodeficiency to mere antibody deficiency

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Null mutations in genes involved in V(D)J recombination cause a block in B- and T- cell development, clinically presenting as severe combined immunodeficiency (SCID). Hypomorphic mutations in the non-homologous end joining gene DCLRE1C (encoding ARTEMIS) have been described to cause atypical SCID, Omenn syndrome, Hyper IgM syndrome and inflammatory bowel disease-all with severely impaired T-cell immunity.

By whole-exome sequencing, we investigated the molecular defect in a consanguineous family with three children clinically diagnosed with antibody deficiency.

We identified perfectly segregating homozygous variants in DCLRE1C in three index patients with recurrent respiratory tract infections, very low B cell numbers and serum IgA levels. In patients, decreased colony survival after irradiation, impaired proliferative response, and reduced counts of naïve T cells were observed in addition to a restricted T cell receptor repertoire, increased palindromic nucleotides in the complementarity determining regions 3, and long stretches of microhomology at switch junctions. Defective V(D)J recombination was complemented by wild-type ARTEMIS protein in vitro. Subsequently, homozygous or compound heterozygous DCLRE1C mutations were identified in nine patients from the same geographic region.

We demonstrate that DCLRE1C mutations can cause a phenotype presenting as only antibody deficiency. This novel association broadens the clinical spectrum associated with ARTEMIS mutations. Clinicians should consider the possibility that an immunodeficiency with a clinically mild initial presentation could be a combined immunodeficiency, so as to provide appropriate care for affected patients.

Insights into lymphoid organs throughout vertebrate evolution

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The ancient endowment of animal defenses with innate immunity molecules and cells was accompanied later in evolution by mechanisms able to discriminate more precisely antigens and to underpin effector, anamnestic and regulatory functions.

The vertebrate lymphocytes acquired self/non-self discrimination mainly through MHC/TCR binding (Gnathostomes), although an apparently convergent evolution led to alternative mechanisms in Agnatha.[4]

Key factors in selection of the "classical" MHC restriction have been the appearance and specialization of primary lymphoid organs for progenitors recruitment, differentiation, selection and maturation.[1]

Neural crest cells of Gnathostomes could migrate into pharyngeal pouches to establish the proper thymic stroma and microenvironments, and orchestrate the fine processes resulting in emigration of self-tolerant naïve T cells. Thymocyte education apparently relies on exposure to self-antigens only, even in the most primitive anatomical arrangements of thymus, exposed on both gill chambers and separated by the water environment only by an epithelial monolayer.

The existence of different antigen-specific T cell subsets (cytotoxic, helper and regulatory) has been already demonstrated in jawed fish and ongoing research is providing hits for a sub-classification and new discoveries.

The thymus apparently provides the primary site for T-lymphopoiesis in all Gnathostomes studied so far, whereas the anatomical sites of primary differentiation of B cells differ remarkably. These sites can be the epigonal and Leydig's organs in adult Chondrichthyes (liver and kidney in embryos), or the kidney in many other ectotherms (Osteichthyes, Amphibians), actively hematopoietic and recalling the histology of bone marrow. This latter appears in Amphibians (largely dependent on the liver for B-lymphopoiesis), but achieves a significant lymphopoietic role only in Sauropsida, that becomes prominent in Mammals.

Indeed, the (cloacal) bursa Fabricii is the main primary organ for B cells in all Aves orders, and the models of B-cell lymphopoiesis differ considerably among mammalian clades, and even species. Within Eutheria, the bone marrow is the main primary site in some adult Rodents and Primates, while in other mammalian species genetic rearrangement of B lymphocyte repertoire occurs in the gut-associated lymphoid tissue (GALT). It is worth considering that this issue is only partially addressed in some mammalian species, and never studied in many. The general assumption holds true that the lymphopoietic potential of the GALT would deserve huge research efforts.[5]

Naïve lymphocytes encounter the epitopes in secondary organs that vary considerably in the different Gnathostome clades, e.g. the spleen (evo novelty), kidney marrow (in ectotherms), lymph nodes (in omeotherms, but "canonical" only in Mammals). The mucosa-associated lymphoid tissues (MALT: gut, respiratory organs, skin) are mostly represented in less evolved Gnathostomes by GALT, that can coordinate efficient local responses but hardly systemic ones, due principally to the lack of lymphoid aggregates and mesenteric lymph nodes, that evolved and differentiated in Amniotes. The organization of secondary lymphoid tissues apparently rose throughout Gnathostome evolution, linked to higher efficiency of adaptive response and associated selection events.[3] The achievement of an omeothermic condition, besides metabolic advantages, certainly posed health risks, and the needs to fight successfully challenging microbes (especially viruses) and higher numbers of transformed cells inside the body. These driving forces promoted the establishment of highly organized, encapsulated structures, where antigen-presenting cells (APC) and lymphocytes would interact properly and efficiently scan the antigens arising from circulating blood (spleen) or lymph (lymph nodes).

However, in both cartilaginous and bony fish the APC already underwent main specializations, culminating in dendritic cells (DC) features documented at cytological, molecular and functional levels, even if their developmental pathways are still undiscovered. Interestingly, at least in rainbow trout MHC II+CD8-α+ DC were detected in the skin, leading to hypothesize also antigen cross-presentation.[2]

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Cellular therapy in transplantation: a dream of good doctor

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T regulatory cells (Tregs) are potent tool in cellular therapy. Due to dominant immunosuppressive activity of these cells in the body, they are considered as a therapeutic agent in transplantation setting, autoimmune and allergic conditions.

Here, we will describe our ongoing clinical studies in the Medical University of Gdańsk in patients after bone marrow transplantation, where cellular therapy with CD4+CD25highCD127- Tregs is administered as a prophylaxis or treatment of graft versus host disease (GvHD) and in diabetes type 1, where CD4+CD25highCD127- Tregs are administered to prediabetic patients as a 'diabetes vaccine'. In addition, we will present a perspective of the local use of Tregs as immunosuppression after pancreatic islets allotransplantation. We will also present and discuss crucial aspects necessary to maintain high quality during sorting, expansion and administration of Tregs to patients.

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The challenge of transplant rejection: from understanding allorecognition to inducing tolerance

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Transplantation has been a very successful approach for the treatment of end stage organ failure. However while immunosuppressive drugs have progressively reduced the incidence of acute rejection and early graft loss, their use has incurred significant costs. Aside from the myriad of toxicities, current therapies have also failed to prevent chronic allograft dysfunction. Despite the enormous success that has been achieved in reducing acute rejection, the average half-life of a cadaveric kidney or heart transplant has changed little and remains approximately 12 years. Two well defined pathways are involved in the recognition of donor-derived major histocompatibility complex (MHC) molecules. In the direct pathway allogeneic MHC molecules are recognized intact on donor-derived dendritic cells (DCs) while in the indirect pathway they are processed and presented as peptides in the context of recipient MHC molecules by recipient DCs. While these two pathways are both initiated following transplantation, the indirect pathway was thought to be the major driver of late graft failure. Recently we have proposed a third pathway of allorecognition, semi-direct, that involves the capturing of intact donor MHC molecules by recipient DCs and links direct and indirect alloresponses at the surface of recipient dendritic cells. Data from mouse transplant models suggest that the semi-direct pathway persists for a long time after transplantation and is amplified by inflammation. This suggests that the direct alloresponse is a long-term threat to the transplant and informs tolerance-promoting strategies.

One of the strategies to induce transplantation tolerance is to either increase the endogenous pool of CD4+CD25+FOXP3+ (Tregs) or to expand Tregs *ex vivo* and inject them back *in vivo*. Tregs play an important role in immunoregulation and have been shown in animal models to promote transplantation tolerance. Phase I trials in bone marrow transplantation have shown that *ex vivo* expanded Tregs have an excellent safety profile, encouraging the broader application of these cells. We have accumulated data in animal models to support the contribution of Tregs to graft survival/tolerance in animal models of skin/heart transplants. We have also demonstrated that Tregs that are specific for the graft are superior to self-specific or polyclonal Tregs. We are now extending this work to the clinic, requiring GMP-compliant cell expansion, and are engaged in two phase 1 trials.

IKBKE maintains a pro-inflammatory loop for intestinal tumor development

Serkan Goktuna

Wnt signaling promotes intestinal cell proliferation but cancer development also relies on the tumor microenvironment. The role of signaling proteins required for the establishment of tumor microenvironment has not been extensively studied. Here, we assessed the role of the pro-inflammatory Ikk-related kinase IKBKE in Wnt-driven tumor development using genetically modified mouse models of intestinal cancer.

IKBKE is activated upon Apc loss and is required for tumor development by limiting TNF- and Caspase 8-dependent apoptosis and by promoting the constitutive activation of Akt, Mek1/2, Erk1/2 and Msk1 kinases in transformed intestinal epithelial cells. As a result, genes encoding pro-inflammatory cytokines and chemokines are upregulated leading to recruitment of tumor-associated macrophages to ultimately promote IL-17A synthesis. IL-17A synergises with commensal bacteria derived products to trigger IKBKE phosphorylation in transformed intestinal epithelial cells and establishes a positive loop further supporting tumor development.

Therefore, TNF-, LPS- and IL-17A-dependent signaling pathways converge to IKBKE for cell survival and for the establishment of an inflammatory tumor microenvironment in the intestine.

Chronic inflammatory microenvironment defines the pro-tumorigenic functions of IFN-γ

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Significant progress has been made in the treatment of patients with melanoma during the last decade, including the approval of new, targeted agents that have provided remarkable responses as well as immunotherapies that provide durable benefit to the small subset of patients that respond. However, the majority of melanoma patients do not respond to either of these therapies, and a large proportion of patients that initially respond ultimately relapse, with a dismal prognosis. Our clear understanding of melanoma molecular identity, to elucidate tumor immunity at a cellular and molecular level, is significant. Thus, we focus on IFN-γ signaling, which had been shown to have both anti- and pro-tumorigenic effects, with particular emphasis on CD74 as a novel target by which to abrogate IFN-γ-related survival mechanisms in melanoma. In our current approach we target to manage chronic low grade inflammation, induced from tissue stress and malfunction, which promotes tumor progression by several mechanisms that IFN-γ regulates.

We have learned from The Cancer Genome Atlas Network (TCGA) efforts that a genomic framework of melanoma classification has potential implications for prognosis and therapy and that may relate to recent advances in immunotherapy. Over the past years, immunotherapy and targeted therapy have dramatically changed the landscape of melanoma treatment. As a result, numerous new treatment options are being inducted in the clinic. Our clear understanding of melanoma molecular identity, to elucidate tumor immunity at a cellular and molecular level, will create significant foundation on combinational treatment approaches. We propose, IFN-γ, which had been shown to have both anti- and pro-tumorigenic activities to be tuned to host's benefit. One potential way achieving this is systemically exploring its role in melanoma tumor environment, which seems to have differential effects on tumor cells versus infiltrating immune cells. The major biological responses to IFN-γ, includes inhibition of proliferation, antiviral responses, immune modulation, and tumor suppression.

Despite the fact that controlled inflammatory response is beneficial, it could become detrimental if dysregulated. Inflammatory responses differ greatly depending on the nature of the instigators. As a result, dysregulated inflammation might be responsible for the chronic inflammatory conditions those are associated with cancer. Melanoma is among highly immunogenic cancers associated with substantial immune cell infiltration and can sometimes exhibit spontaneous regression. It is now also accepted that it might be affected by cross-talk with the immune response, which could lead either anti-tumor immune cell activation or to immune response suppression to support cancer cell survival and metastasis.

CD74/MIF/CD44 axis plays a role on specific biological activities related directly to cancer growth or contributing towards a microenvironment favoring cancer progression. It has been reported each member of this axis acts a key part in malignancy by contributing to the reception of a broad variety of microenvironmental components, including extracellular matrix constituents, growth factors and cytokines. Therefore, novel therapeutic approaches, involving inhibition of both MIF activity and CD44 binding, would be most ideal tactic to control downstream effects of this pathway which seems mostly favoring cancer progression. While achieving this control, it is equally important to keep MHC II/CD74 complexes active for peptide presentation to CD4+ T helper cells.

Cancer-related inflammation

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The development of many tumor types is associated with chronic infection and inflammation. At the same time, tumor persistence and progression are itself the cause of inflammatory processes. Constantly ongoing cell death, tissue trauma and release of so-called danger-associated molecules induce a process referred to as cancer-related inflammation. This inflammation, together with progressive tumor growth, may have local, but also systemic effects in the tumor host.

In our group we study the role of neutrophils and myeloid-derived suppressor cells (MDSC) as primary immunologic mediators of cancer-related inflammation. We also investigate the immunomodulatory activity of mesenchymal stromal cells (MSC) isolated from human cancer tissues.

We have identified and characterized pathways of recruitment and activation of tumor-associated neutrophils in head and neck cancer. Subsequently, we have elucidated feedback mechanisms exerted by neutrophils on tumor cells, which resulted in the generation of a metastatic tumor cell phenotype. By using tomato fluorescence under the control of a Ly-6G promotor, a novel mouse model now allows *in vivo* live cell imaging of tumor associated neutrophils.

MDSC are a heterogeneous population of immature and mature myeloid cells expanded in the tumor host. MDSC were first identified in mice and comprise monocytic and granulocytic subsets. Immunosuppression, best demonstrated for T cells, is a hallmark of MDSC function. We have developed immunomonitoring protocols for human MDSC and have characterized cell biological functions and clinical relevance of granulocytic MDSC isolated from the peripheral blood of patients with head and neck cancer.

Collectively, these studies suggest that tumors exploit the inflammatory and immunoregulatory activity of neutrophils and MDSC to promote tumor progression and metastatic spread.

DOCK8 deficiency: cellular and molecular mechanisms

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The Dedicator of cytokinesis 8 (DOCK8) is a guanine nucleotide exchange factor (GEF) that coordinates the actin cytoskeleton response to mitogenic and chemokine signals, most notably by regulating the activity of the small GTP binding protein cell division cycle 42 (CDC42).[1] Studies that the late Professor Dr. Işıl Berat Barlan played a critical role in their initiation led to the discovery of DOCK8 deficiency due to loss-of-function deletions and mutations in DOCK8 to underlie the majority of cases with the autosomal recessive form of the hyper IgE syndrome (AR-HIES).[2-4] The latter is a combined primary immunodeficiency disease (PID) that is characterized by susceptibility to viral, bacterial and fungal infections and which disproportionately affects Turkish children. Its manifestations include recurrent sinopulmonary infections, cutaneous and systemic viral infections, mucocutaneous candidiasis, atopic manifestations including eczema, food allergy and asthma with hyper IgE and eosinophilia, early onset autoimmunity and virally-driven malignancies.[5,6] DOCK8 deficiency broadly affects both innate and adaptive components of the immune system including T cells, B cells NK and NKT cells, dendritic cells and innate lymphoid cells. Almost all patients with DOCK8 deficiency either lack or are profoundly deficient in DOCK8 protein expression. Nevertheless, by studying a unique DOCK8-deficient subject with a point mutation in the GEF catalytic center that abolishes its activity while preserving protein expression, it could be shown that abrogation of DOCK8 GEF activity recapitulated all key attributes of DOCK8 deficiency.[7]

The phenotype of DOCK8 deficiency overlaps with that of the autosomal dominant form of the hyper IgE syndrome (AD-HIES), due to heterozygous loss of function mutations in STAT3. Both diseases exhibit a hyper IgE phenotype, and susceptibility to bacterial and candidal infections. AD-HIES is associated with failure of Th17 cell differentiation due to ineffective STAT3 activation. Our studies revealed a similar defect in Th17 differentiation in DOCK8-deficent subjects, related to impaired STAT3 activation by cytokines in T cells. DOCK8 constitutively associates with STAT3 and augments its activation by cytokines in a CDC42-dependent manner.[7] Furthermore, DOCK8-STAT3 complexes translocate to the nucleus, enabling in the process STAT3 transcriptional function. These studies outline both common and divergent mechanisms operative in distinct genetic forms of HIES.

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NK cell-based approaches in cancer immunotherapy

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About a century after the initial proposition that the immune system has the capacity to fight against tumors, evading destruction by immune cells is now well recognized as a hallmark of cancer. The development of any malignancy is under close surveillance by members of the immune system, but malignant cells find ways to escape immunological responses. Natural Killer (NK) cells are members of the lymphocytic lineage that have an essential role in immunosurveillance against tumors and infected cells. The potential of using NK cells for cancer immunotherapy continues to inspire research on various perspectives ranging from *ex vivo* expansion to genetic modification for maximizing and directing anti-tumor activity.

This research investigates the feasibility and potential of *ex vivo* expanded and/or genetically modified NK cells for cancer immunotherapy. We have optimized a GMP-compatible system that has the capacity to expand polyclonal and highly cytotoxic NK cells using an automated bioreactor. Furthermore, we have investigated methods for efficient genetic modification of NK cells for cancer immunotherapy purposes. Genetic modification of NK cells can be carried out with lentiviral vectors, however the gene transfer efficiency is markedly lower when compared to other cells of hematopoietic origin. We have hypothesized that the innate antiviral defense systems in NK cells restrict lentiviral vector integration and the inhibition of these signals can lead to better gene delivery. Our efforts have resulted in an optimized stimulation and genetic modification process for NK cells that greatly enhances lentiviral gene delivery.

Finally, we have optimized the production of NK cell-derived exosomes that show cytotoxic activity against tumor cell lines. The effect of different cell-culture media and external cytokine stimuli are being tested for enhancing cytotoxic exosome yield and functionality. Our results show that NK cell-derived exosomes can be functionally purified and display a high level of cytotoxic activity against K562 cells, showing a promise for further development into a possible off-the-shelf approach in cancer immunotherapy.

Taken together, the work presented here aims to bring us closer to optimal *ex vivo* manipulation and genetic modification of NK cells for cancer immunotherapy.

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Subsets of human natural killer cells and their regulatory effect

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Natural killer (NK) cells represent a distinct lymphocyte population with extensive cytolytic activity with a variety of other functions, including regulation of hemopoiesis, suppressor functions and immunoglobulin production. Recently, more and more evidence has been obtained that NK cells also display a potent regulatory function by secreting various cytokines or cell-to-cell contact and thus regulate innate and adaptive immune responses and maintain immune homeostasis.

NK cells are a subset of lymphocytes that principally participate in innate immunity but may also have important roles in determining the outcome of the adaptive immune responses. In human they are phenotypically defined as CD3-CD56+ lymphocytes, and the major functional properties of NK cells are cytotoxicity, cytokine production and contact dependent co-stimulation.

NK cell regulatory capabilities mediated by cytokines and chemokines release, they exert their activity by producing high amount of IFN-γ, that activates a strong inflammatory response. Indeed, other than IFN-γ, NK cells are able to produce many other important cytokines and chemokines, including myeloid differentiation and activation factors such as IL-3, GM-CSF, TNF-α, IL-5, IL-13, IL-10 and chemokines such as MIP-1, RANTES and IL-8.

Similar to the Th1 and Th2 subsets of CD4+ T cells, NK cells are also divided into NK1 and NK2 subpopulations according to the profile of cytokine secretion. Our and other results showed that in the presence of IL-12 or IL-4, human NK cells can differentiate into NK cell subsets secreting distinct cytokine patterns similar to T cells. NK cells grown in IL-12 (NK1) produce predominantly IFN-γ, whereas NK cells grown in IL-4 (NK2) produce IL-5 and IL-13. Although these NK cell subsets do not differ in cytotoxic activity, NK1 cells express higher levels of cell surface CD95 (Fas) antigen than NK2 cells and are more sensitive to antibody or chemically induced apoptosis. It has been showed that the type 2 cytokines produced by NK2 cells are dominant in asthma and tumor microenvironment anda re involved in pathogenesis of asthma and cancer.

The IL-10- and TGF-β-secreting NK3 type cells also play major roles in immune regulation and promote transplant and pregnancy tolerance. Similar to suppression of both cytokine production and antigen-specific proliferation of Th1 and Th2 cells by IL-10, our results showed that IL-10 secreting NK cells suppress both allergen-stimulated T cells and PPD-stimulated T cells, whereas IFN-γ secreting NK cells did not show any suppression. The findings suggest that *in vivo* existence of a regulatory NK cell subset, which indeed may play an immune regulatory and supressor role.

Recently, mucosa-associated IL-22 producing NK cells have been described and are called NK-22 cells. Although activated CD4+ T cells and NK cells secrete IL-22, this cytokine acts exclusively on certain tissue cells. IL-22 play an important role in pathogen defense, wound healing, and tissue re-organization, which can have both proinflammatory and anti-inflammatory capacities. This cytokine is also secreted by Th17 cells, natural killer T (NKT) cells, and γδ T cells.

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ORAL PRESENTATION

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[S-0009]

[Immunity to Infection: New Trends and Developments]

The associations between a gene polymorphism (IL-2 and IL-4) and cytokines in patients with recurrent pulmonary tuberculosis on standard chemotherapy

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Background and Objective: To study the influence of gene polymorphism (IL-2 and IL-4) and cytokines in patients with recurrent pulmonary tuberculosis (RPTB) on standard chemotherapy.

Methods: The study comprised 130 individuals in Kharkiv region of Ukraine including 100 patients RPTB (group 1) and 30 healthy donors (group 2). Serum levels of cytokines IL-2 and IL-4 were evaluated by ELISA. Measurements on serum samples of patients were conducted prior or during first days after admission to the hospital and after 2 months on standard anti-mycobacterial therapy. Investigations of gene polymorphisms of these cytokines were performed using restriction analysis of the amplification products of specific regions of the genome. Two polymorphic variants were examined: T-330G region of IL-2 gene and promoter region C-589T of IL-4. All patients received standard TB drugs: Isoniazid (0.3 g); Rifampicin (0.6 g); Pyrazinamide (2 g); Ethambutol (1.2 g) and/or Streptomycin (1 g).

Results: In the 1st group the levels of IL-4 and IL-2 were 9.55±0.24 pg/L and 39.44±0.71 pg/L, while in 2nd group these values were 29.99±1.27 pg/L and 21.60±0.80 pg/L respectively ($p<0.05$). Among patients with RPTB the heterozygous genotype was most prevalent; 74% (n=74) for IL-2 and 61%(n=61) for IL-4. The homozygous genotype was accordingly less common: 26% (n=26) and 39% (n=39), of which 18% (n=18) and 21% (n=21) of patients had mutation and remaining had normal homozygote genotype, i.e., 8% (n=8) and 18% (n=18) for IL-2 and IL-4 respectively. In contrast, most of healthy donors had normal homozygous genotype with 60% (n=18) and 56.66% (n=17) with low frequency of mutations; 16.66% (n=5) and 23.34% (n=7) and heterozygous genotype 23.34% (n=7) and 20% (n=6)for IL-2 and IL-4 genes respectively. Following a 2 month treatment, there was a significant reduction of cytokine levels in the IL2 (29.59±0.55) pg/L and increased in the IL4 (16.68±0.44) pg/L, when compared to the beginning of therapy and after 2 months ($p<0.001$).

Conclusion: Compared to healthy controls patients with RPTB had significantly lower levels of serum IL-4 and high-IL-2. This coincided with greater frequency of heterozygous polymorphism C-589T and T-330G genes of IL-4 and IL-2. Further studies are warranted whether higher rate of recurrent TB has a causal immunogenetic relationship to allelic polymorphism of genes encoding for IL-2 and IL-4. Standard 2-month TB therapy results in reversal of inflammation characterized by decrease in IL-2 and increase of IL-4 to the levels comparable to healthy donors. IL-4 and IL-2 are immune correlates of treatment outcome and can help to identify better strategy for TB management. TB chemotherapy may have immunomodulatory effect of anti-inflammatory nature.

Keywords: Tuberculosis; cytokines; interleukin-2; interleukin-4; gene polymorphism; treatment of tuberculosis.

[S-0028]

[Tumor Immunology & Molecular Markers]

ATP-binding cassette transporter G1 (ABCG1) is a novel mediator of tumor immunity

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ATP-binding cassette transporter G1 (ABCG1) is a member of the ABC transporter family that regulates cellular cholesterol homeostasis in the cell. Herein, we report a role of ABCG1 as a novel mediator of tumor immunity. Abcg1-/- mice fed a Western diet showed dramatically reduced subcutaneous MB49-bladder carcinoma and B16-melanoma tumor growth compared to control. Abcg1-/- mice also showed diminished spontaneous tumor metastasis and prolonged survival. Selective deletion of ABCG1 in myeloid cells, but not in T cells, reduced tumor growth *in vivo*, demonstrating that the observed effects were mediated through myeloid cell-intrinsic mechanisms. Abcg1-/- mice on Western diet displayed decreased macrophage frequency due to enhanced apoptosis and a shift of the remaining macrophages from a tumor-promoting M2 to a tumor-fighting M1 phenotype within the tumor. Furthermore, *in vitro* polarization studies revealed that Abcg1-/- macrophages exhibited an intrinsic bias towards M1 polarization with increased production of TNF-α, nitric oxide, MHC class II and CD86 and decreased expression of Arg1, Mrc1 and Fizz1. M1 bias of Abcg1-/- macrophages was associated with enhanced NF-κB activation and direct cytotoxicity for tumor cells *in vitro*. Overall, our results show that absence of ABCG1 inhibits tumor growth through modulation of macrophage survival and phenotype within the tumor. This study identifies the cholesterol transporter ABCG1 as a novel mediator of tumor immunity and provides a novel link between cholesterol homeostasis and cancer.

Keywords: ABCG1; cholesterol; tumor; macrophages.

[S-0029]

[Immunity to Infection: New Trends and Developments]

Phenotypic heterogeneity in patients with the same IL12RB1 mutation: screening of 3 families with patients with BCG infection

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Mendelian susceptibility to mycobacterial disease (MSMD) is a rare disorder which is characterized by susceptibility to infections with weakly virulent mycobacteria, including Mycobacterium bovis and Bacille Calmette-Guerin (BCG). These patients are also susceptible to infections with Salmonella and M. tuberculosis. Nine disease-causing genes have been described (IFNGR1, IFNGR2, STAT1, IL12B, IL12RB1, NEMO, ISG15, IRF8, and CYBB). Defect in IL12RB1 gene is the most common cause of the disease.

We screened 3 families with patients with BCG infection, a feature compatible with MSMD, for IL12RB1 deficiency. First we screened 3 probands and 18 family members by flow cytometry (the surface expression of IL12Rβ1 was studied on activated lymphocyte) than Sangers sequencing.

In 11 individuals surface expression of IL12Rβ1 was absent (<1%). Among these only 5 had clinical features compatible with MSMD and all were found to have homozygous c64+1G mutation. Two siblings, two parents and two grandparents without surface expression of IL12Rβ1 were asymptomatic, among those only one sibling had homozygous c64+1G mutation while the rest of the family members were heterozygous both for the mutation and Arg156His polymorphism (rs11575926) which allele frequency is 0.79. It seems that the antibody we use cannot detect IL12Rβ1 with Arg156His polymorphism suggesting that the epitope recognized by the antibody to be around the position of the polymorphism.

Among the 6 affected (one died before family screening was performed) from 3 families carrying the same mutation the severity of the infections varied considerably, from transient axillary drained BCG lymphadenitis without any further infections to severe disseminated BCG infection. One patient who was BCG vaccinated had only mild oral moniliasis.

In conclusion: Testing with two different antibodies may be better for screening by flow cytometry. There is a wide range of clinical presentation in patients with the same mutation and even within the same family. The penetrance of MSMD in IL-12Rβ1 deficiency seems to be incomplete for BCG infection. So even healthy siblings of the proband should be investigated for the defect.

Keywords: IL12RB1; flow cytometry; sequence; mutation.

[S-0048]

[Tumor Immunology & Molecular Markers]

Cancer associated fibroblasts display phenotypic and functional features that resemble circulating fibrocytes which constitute a novel subset of MDSCs

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The studies that have investigated the nature of the cells and molecules responsible for the functional insufficiency of the tumor infiltrating T cells focus on several components; however, the contribution of stromal cellular elements has not yet been well established. Fibroblasts, one of the most abundant cell types found in the stroma, turn into cancer associated fibroblasts (CAFs) and myofibroblasts in the tumor microenvironment. Tissue fibroblasts have previously been shown to have effects on T lymphocyte functions. However, studies investigating the effects of cancer associated fibroblasts on T cells are limited in the literature. Circulating fibrocytes represent a novel MDSC subset and they take part in the tumor immune escape. These fibrocytes display a surface phenotype similar to non-monocytic MDSCs (CD14-CD11chiCD123-) and also show immunomodulatory roles. Since the effector functions of fibrocytes are carried out as tissue fibroblasts, we aimed to evaluate if CAFs demonstrate similar molecular / gene expression patterns and functional characteristics to the circulating fibrocytes. N-Nitroso-N-Methylurea (NMU) induced breast cancer model was utilized to obtain CAFs. Possible DNA damages due to NMU injections were evaluated by Comet Assays. Fibroblasts and CAFs were isolated from cancerous and healthy breast tissues, using an enzymatic protocol with collagenase and hyaluronidase. Isolated CAFs and NFs were immunostained to investigate differential expressions of surface markers such as α-Smooth Muscle Actin (αSMA) and vimentin, in order to distinguish CAFs from NFs. CAFs and NFs were evaluated for their surface marker expressions by flow cytometry and for gene expression profiles by gene set enrichment analysis. Cocultures of CAFs and NFs with PBMCs were performed and CFSE proliferation assays were used for functional analyses. Levels of DNA damage of tumor bearing animals were similar to control levels about 2 months after injections. CAFs were spindle shaped cells unlike their circulating counterparts and had significantly higher levels of αSMA than NFs. CAFs did not express CD80, granulocytic or neutrophilic markers.

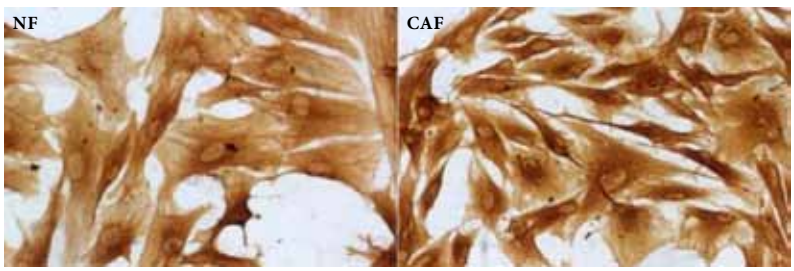

Figure 1. Vimentin stained NF and CAF cells from representative samples (original magnification x200 under light microscope and visualized by DAB).

Their MHC-II expression was lower than NFs. CAFs expressed the myeloid marker CD11b/c; however, its expression was lower than that on their circulating counterparts. They appeared to have developed in a milieu containing T helper 2-like cytokines. CFSE proliferation assays showed the immunosuppressive effects of CAFs similar to their blood-borne counterparts. In summary; CAFs resemble the circulating fibrocytes that were reported to represent a novel MDSC subset, in terms of phenotypic and functional features.

Authors declare no conflict of interest.

Keywords: Breast cancer; cancer associated fibroblast; chemical carcinogenesis; T cell; tumor immunity.

[S-0060]

[Regulatory Cells of Immune System]

S1P1 deletion differentially affects TH17 and regulatory T cells

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Sphingosine-1 phosphate receptor 1 (S1P1) is a G-protein coupled receptor critical for the egress of T and B cells out of lymphoid organs. Although S1P1 agonists (such as fingolimod) are currently used for the treatment of multiple sclerosis (MS) little is known how S1P1 signaling regulates Th17 and Treg cell homeostasis. To study the impact of S1P1 signaling on Th17 and Treg biology, we specifically deleted S1P1 in Th17 and Treg cells using IL-17ACre and Foxp3Cre mice, respectively. Deletion of S1P1 in Th17 cells conferred resistance to experimental autoimmune encephalomyelitis (EAE) characterized by reduced Th17 cell distribution across peripheral organs and diminished Th17 cell generation. On the other hand, permanent deletion of S1P1 in Treg cells resulted in autoimmunity and acute deletion rendered mice more susceptible to EAE. Importantly, our study revealed that S1P1 not only regulated the egress of Treg cells out of lymphoid organs and subsequent non-lymphoid tissue distribution but also their phenotypic diversity. Most of the Treg cells found in S1P1-deficient mice had an activated phenotype and were more prone to apoptosis, thus converted to effector Treg. The comparison of Treg cells obtained from MS patients treated with fingolimod to those treated with other oral drugs confirmed the switch of Treg cells into effector memory phenotype. Our results provide novel insight into the functions of S1P1 and potential impact of long term fingolimod use on Th17 and Treg cell biology and general health in MS patients.

Keywords: Th17; Treg; S1P1; EAE; autoimmunity.

[S-0062]

[Tumor Immunotherapy]

Uroplakin 3A derived peptide vaccination reduced tumor growth in bladder cancer mice model

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Introduction: Uroplakin 3A (UPK3A) is a well-chosen target to develop autoimmune response against bladder cancer since the antigen is also expressed in bladder tumor. In previous study, we developed T-cell mediated bladder specific autoimmunity by targeting bladder-specific UPK3A protein. In this study, we analyzed immune response in BALB/c mice after immunization with UPK3A 65-84 peptide its ability to reduce bladder tumor growth.

Material and Method: Keyhole Limpet Hemocyanin (KLH) conjugated peptide was used with either CFA (Complete Freund's Adjuvant) or CpG (Cytosine-phosphate-guanine) as effective adjuvants. Bladder cancer model in mice was formed after OH-BBN administration to the mice for 12 weeks. Immune response evoked by UPK3A 65-84 peptide with different adjuvants was compared with the detection of immune cells by flow cytometer. The apoptosis induction capability of this vaccination in tumor tissue was also analyzed.

Results and Discussion: We observed that CpG combined with KLH conjugated peptide (UPK3A 65-84) promotes robust immune response via activation of higher immune cells (CD4+ T cell, CD8+ T cells, NK cells CD11b) than KLH conjugated peptide with CFA. CFA or CpG as an adjuvant combined with KLH conjugated peptide significantly reduced tumor growth compared to control groups in mice with bladder.

Conclusion: This peptide is well-chosen target for the development of bladder cancer immunotherapy. Accordingly, UPK3A 65-84 peptide vaccination could provide safe and effective protection against the development of bladder cancer.

Note: This work was supported by The Scientific and Technological Research Council of Turkey (TUBITAK), Grant Number: 113S927.

Keywords: Bladder cancer; uroplakin 3A; cytosine-phosphate-guanine; Th1/Th2 immune response.

[S-0064]

[Transplantation Immunology]

Donor-recipient killer immunoglobulin like receptor (KIR) gene-gene matching improves post-transplant outcomes in HLA identical sibling hematopoietic cell transplantation: matching reduces frequency of relapse and graft versus host disease

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Introduction: Natural killer (NK) cell activation or inhibition, in addition to HLA, is involved in graft versus host disease (GvHD) and graft versus leukemia effects. KIRs can activate (aKIR) or inhibit (iKIR) natural killer cell mediated cytotoxic activity. This prospective study aims to evaluate the effects of both aKIR and iKIR matching status in a homogenous young patient population being transplanted from sibling donors.

Patients and Method: Ninety six patients that were performed an HLA full-matched and T-cell replete sibling transplantation between 1994 and 2008 were included. All patients had a myelogenous malignancy, acute myeloid leukemia or chronic myleogenous leukemia. Low/intermediate resolution molecular typing was performed for HLA-A, -B, -C, -DRB1 loci using sequence specific primers (SSP, Olerup) or the Luminex technology (Tepnel). All patients and donors were tested for 15 functional KIR genes, namely KIR2DL1, 2DL2, 2DL3, 2DL4, 2DL5a, 2DL5b, 3DL1, 3DL2, 3DL2, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5 and 3DS1 by using KIR genotyping SSP Kits (Pel-Freeze, Dynal Biotech, USA and Olerup, Sweden). The absence of any of the KIR genes present on their partner for inhibitory or activating genes were accepted as a mismatch. Five transplant outcomes were analyzed: overall survival at five years (OS), disease-free survival at 5 years (DFS), relapse rate at 5 years (RR), cumulative incidence of aGvHD and cGvHD. OS, DFS and RR were calculated from the time of transplant. SPSS software version 20.0 was used for analysis.

Results: The median age of patients was 36 (range 14-63). Matching was noted for aKIR (n=34) or iKIR (n=38). Following univariate analysis, aKIR-mismatching was found to increase relapse rate (39% vs. 14%, p=0.01) and to decrease DFS (77% vs. 47%, p=0.008). Matching for aKIR or iKIR reduced cGvHD (58% vs. 77%, p=0.04; 55% vs. 81%, p=0.008). cGvHD was further reduced when both aKIR and iKIR were matched (50% vs. 84%, p=0.03). In multivariate analysis, aKIR-mismatching was persistently associated with increase in relapse (HR: 3.93, p=0.01) and poor DFS (HR: 0.46, p=0.04).

Discussion: Our findings contribute to previous reports with results confirming aKIR-matching to be associated with less myeloid leukemia relapse. Our original contribution is rather than the specific KIR type or the number of KIRs, matching for aKIR reduces relapse. Furthermore we found matching for aKIR and iKIR to reduce cGVHD incidence. In conclusion, donor-recipient aKIR and/or iKIR-matching influence transplant outcomes positively and may point to a residual recipient natural killer cell activity.

Keywords: KIR genes; hematopoietic stem cell transplantation; relapse; graft versus host disease.

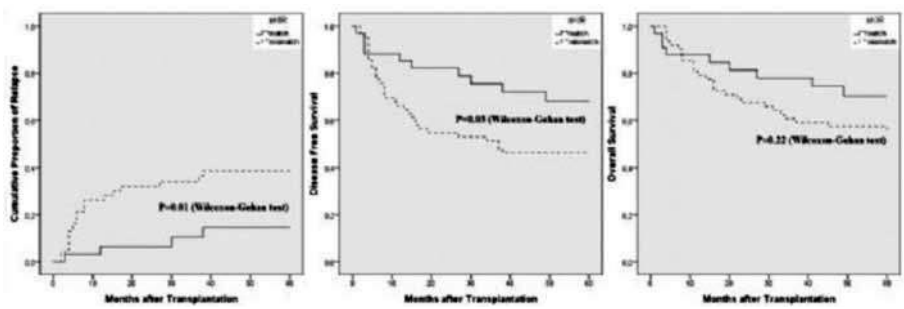

Figure 1. Life tables for RR **(a)**, DFS **(b)** and OS **(c)** according to aKIR-matching status.

[S-0071]

[Emerging Issues in Adaptive Immune System]

Accumulation of AID in immunoglobulin variable genes in the absence of Topoisomerase I

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Activation-induced deaminase (AID) is a DNA cytosine deaminase that diversifies immunoglobulin genes in B cells. Recent work has shown that RNA polymerase II (Pol II) accumulation correlates with AID recruitment. However, a direct link between Pol II and AID abundance has not been tested. We used the DT40 B-cell line to manipulate levels of Pol II by decreasing topoisomerase I (Top1), which relaxes DNA supercoiling in front of the transcription complex. Top1 was decreased by stable transfection of a short hairpin RNA against Top1, which produced an accumulation of Pol II in transcribed genes, compared to cells transfected with sh-control RNA. The increased Pol II density enhanced AID recruitment to variable genes in the λ light chain locus, and resulted in higher levels of somatic hypermutation and gene conversion. It has been proposed by another lab that AID itself might directly suppress Top1 to increase somatic hypermutation. However, we found that in both AID(+/+) and AID(-/-) B cells from DT40 and

mice, Top1 protein levels were identical, indicating that the presence or absence of AID did not decrease Top1 expression. Rather, our results suggest that the mechanism for increased diversity when Top1 is reduced is that Pol II accumulates and recruits AID to variable genes.

Keywords: Activation-induced deaminase (AID); DT40; gene conversion; RNA polymerase II; somatic hypermutation; topoisomerase I.

[S-0075]

[Cellular and Molecular Targets in Rheumatoid Arthritis and Autoimmune/Autoinflammatory Diseases]

Src family kinases dependent STAT3 activation is involved in LPS-induced cytokines production in PBMC of patients with Behçet's disease

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Behçet's disease (BD) is a chronic inflammatory disorder characterized by recurrent oral and genital ulcers, uveitis and skin lesions. The pathogenesis of the disease remains poorly understood. Immunological abnormalities are implicated in the BD pathophysiology. Aberrant production of some cytokines may play an important role in the pathogenesis of BD. Revealing a key signalling regulatory mechanism involved in cytokines production is critical for understanding of the pathogenesis of BD. TNF-α is a pro-inflammatory cytokine that acts as a major mediator in the pathogenesis of inflammatory diseases. IL-10 is an anti-inflammatory cytokine that regulates excessive production of inflammatory cytokines. Src family kinases (SFKs) are implicated in signalling pathways induced by lipopolysaccharide (LPS), but the role of SFKs in LPS-induced cytokines production has not been investigated in BD patients. In present work, we addressed the role of SFKs in LPS-induced inflammatory responses in peripheral blood mono nuclear cells (PBMC) of active BD patients.

20 active BD patients and 20 healthy individuals were included in this study. PBMC were isolated from venous blood and cultured for further studies. PBMC were treated with PP2, a selective inhibitor of SFKs, before LPS stimulation. The effect of the chemical inhibition of SFKs on LPS-induced production of TNF-α and IL-10 in supernatants of cultured PBMC were examined by ELISA. Moreover, the role of SFKs on LPS-induced signalling pathways, including MAPK and STAT3 was investigated by Western Blotting.

Our results showed that blocking SFKs activity by PP2 treatment inhibited LPS-induced TNF-α and IL-10 productions in PBMC of active BD patients. The implication of STAT3 activity in inflammatory responses has been shown to be mediated through SFKs activity. Intriguingly, we found that inhibition of SFKs by PP2 led to a significant increase in LPS-induced pERK MAPK phosphorylation. However, LPS-induced pJNK and p38 MAPK phosphorylation were not significantly affected by inhibition of SFKs activity.

In Conclusion, this study demonstrates that SFKs activity is absolutely required for LPS-induced STAT3 activation leading to TNF-α and IL-10 productions in PBMC of active BD patients. Taken together, SFKs might be a future drug target for BD treatment.

This study was supported by the TÜBİTAK (Scientific and Technical Research Council of Turkey) under the grant 213S016.

Keywords: SFKs; BD; cytokine; STAT3; PBMC; LPS.

[S-0086]

[Tumor Immunotherapy]

The immune escape in ovarian cancer: role of epigenetic mechanisms in suppression of positive costimulatory molecules in chemoresistant ovarian cancer cells

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The immunological response against cancer is a critical balance between immune-activating and immune-suppressing mechanisms. Ovarian cancer creates a suppressive microenvironment to escape immune elimination. However, the molecular mechanisms to escape immune elimination are poorly understood. Tumor cells escape immune responses by downregulating genes that are essential for effective antitumor immunity such as genes involved in presentation of antigens to T cells, stimulation of T cells, and susceptibility to apoptotic signals. Members of the tumor necrosis factor (TNF) receptor superfamily 4-1BBL and OX-40L are expressed on antigen presenting cells and have been reported to be expressed on tumor cells. Interaction between these ligands with their cognate receptors, 4-1BB and OX-40 promotes T cell division, survival, cytokine production and effector cell activity. This study demonstrates that expression of positive costimulatory molecules, OX-40L and 4-1BBL, is suppressed while expression of immunosuppressive molecule programmed death ligand-1 (PD-L1) is enhanced in A2780-AD chemoresistant ovarian cancer cells compared to parental chemosensitive A2780 cells. Here, the molecular mechanisms of silencing of OX-40L and 4-1BBL expressions were investigated in chemoresistant A2780-AD ovarian cancer cells. The suppression of OX-40L and 4-1BBL are due to DNA hypermethylation and histone deacetylation, two important mechanisms that contribute to gene silencing during cancer progression. We identify important epigenetic regulators, histone deacetylase 1/3 (HDAC1/HDAC3) and DNA methyltransferase 1 (DNMT1), that exhibit aberrant association with OX-40L and 4-1BBL promoters in chemoresistant ovarian cancer cells. Knockdown of HDAC1 or DNMT1 expression, and pharmacological inhibition of DNMT or HDAC enzymatic activity, significantly increases OX-40L and 4-1BBL expressions in chemoresistant cells. This study suggests that loss of histone acetylation and accumulation of DNA methylation correlates with suppressed expression of OX-40L and 4-1BBL in chemoresistant ovarian cancer cells. This study marks the first report of the regulation of these two molecules by histone deacetylation and DNA methylation in chemoresistant ovarian cancer cells.

Keywords: Ovarian cancer; immune suppression; costimulatory molecules; DNA methylation; histone acetylation.

[S-0090]

[Emerging Issues in Adaptive Immune System]

Effects of gender differences and sex hormones on mouse bone marrow cell populations

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Introduction: Thymus and bone marrow are the two critical primary lymphoid organs that lose their capacity during aging. Bone marrow (BM) produce the T cell progenitors and they migrate to thymus for selection and maturation. Therefore, thymic T cell production capacity is affected by changes in bone marrow cell populations. It is known that females have lower thymic involution rates than males. In our study, we analyzed the effects of gender differences, sex hormones on bone marrow stem progenitor cells.

Materials and Method: C57BL/6 mice were divided into six experimental group, each group was containing seven mice. Seven days old male and female mice bone marrow, thymus and peripheral blood were collected for first 2 groups. Same samples were collected from other groups at the end of the three months. Other groups were fed for 3 months, one male and one female group were fed with normal chow and untreated, while a female group were treated with testosterone and another female group were treated with letrozole for inhibition of estrogen synthesis. Filtered bone marrow was analyzed with flow cytometry for Lin-/lowSca1+CD117+CD135-haematopoietic stem cells (HSC), Lin-/low CD117 high CD135+Sca1+CD90-multipotent progenitor (MPP) cells and Lin-Scalow CD117 low CD127+CD135+ common lymphoid progenitor (CLP) cells.

Results: Ratio of MPP and CLP cells in the bone marrow were higher for 7 days and 3 months old females when compared to same age males. MPP cells in the 3 months old female group was 1,5 fold higher than those of 3 months old male group, CLP cells in the 3 months old females was three fold higher than those of 3 months old male group. Letrozole treatment in 3 months old female group decreased the ratio of MPP and CLP cells in total bone marrow cells, ratio of MPP and CLP cells in BM cells significantly increased in testosterone treated 3 months old female group.

Discussion: Testosterone treatment in female mice, increased the number of MPP and CLP cells in bone marrow while suppression of estrogen synthesis decreased the number of same cells. Therefore, testosterone is not responsible of the lower numbers of MPP and CLP cells in bone marrow of 3 months old males, estrogen deficiency seems as the main actor in this situation.

Keywords: Bone marrow; estrogen; gender; haematopoietic stem cells; progenitor cells; testosterone.

[S-0105]

[Novel Discoveries in the Innate Immune System]

Modulating the differentiation of human monocytes into immunosuppressive macrophages via TLR1/2 signaling

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Monocytes can mature into two functionally distinct populations: pro-inflammatory M1 and immunosuppressive M2 macrophages (hereafter M1 or M2). M1 provide protection against infectious pathogens but if uncontrolled can cause inflammation and autoimmune disease. M2 down-regulate inflammatory responses and help maintain homeostasis. We previously reported that the TLR1/2 dual agonist PAM3 can trigger monocytic myeloid-derived suppressor cells (mMDSC) to mature into M2. We now examine the effect of PAM3 on classical monocytes. Towards that end, CD14hiHLA-DRhi cells were isolated from elutriated monocytes of 25 healthy donors. PAM3 was unique among a panel of TLR agonists tested in being able to support the differentiation of HLA-DR+ monocytes into phenotypically M2 macrophages. The effect of PAM3 was compared to M-CSF, a growth factor that supports the generation of immunosuppressive macrophages. Lower expression of CD16, PD-L2, SR-A1 and DC-SIGN by PAM3-generated macrophages suggested that PAM3 is slightly less efficient than M-CSF in driving M2 differentiation. Yet macrophages generated in the presence of either PAM3 or M-CSF were functionally equivalent in their suppression of autologous T cell proliferation. As addition of anti-M-CSF Ab had no effect on PAM3-dependent M2 polarization, we conclude that PAM3 and M-CSF mediate M2 polarization via independent routes. To clarify the pathways by which immunosuppressive M2 arise, we compared the regulatory networks activated during the course of their differentiation. PAM3 and M-CSF both utilized an NF-κB dependent signaling mechanism. Blocking this pathway prevented M-CSF-induced monocyte differentiation and significantly reduced PAM3-dependent differentiation. However PAM3 also induced monocyte differentiation via an IL-6-dependent pathway that was largely absent from M-CSF driven cultures. These studies demonstrate that a TLR agonist induces monocytes to mature into immunosuppressive M2 macrophages and identifies conserved, targetable pathways involved in that process. M-CSF has pleiotropic effects on myelopoesis and tissue-resident macrophages and thus cannot be used therapeutically to generate M2 macrophages in patients with autoimmune disease. Current findings suggest that PAM3 may provide a therapeutic alternative, given its ability to modulate monocyte differentiation.

Keywords: M2 macrophages; PAM3; M-CSF.

[S-0111]

[Immunodeficiencies: Emerging Molecular & Cellular Therapies. Remembering Prof. Isıl BARLAN]

Personalized therapy for X-linked agammaglobulinemia (XLA) by oligonucleotide-induced splice-correction

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An important percentage of disease causing mutations affects pre-mRNA splicing which is controlled by sequence elements within the exons and introns. Mutations disrupting the sequence elements commonly result in exon exclusion or cryptic exon inclusion named as pseudoexon. The result is typically a frame-shift in the pre-mRNA causing production of partially functional or a defective protein. A common way to correct splicing mutations is to use antisense oligonucleotides (AONs) that are short complementary sequences that bind to the pre-mRNA and re-direct the splicing.

X-linked agammaglobulinemia (XLA) is an inherited immunodeficiency resulting from mutations in the BTK gene severely impairing B cell development. BTK has a crucial role in B cell development and the absence of BTK introduces a developmental block. A prominent percentage of splicing mutations affect pre-mRNA splicing in XLA. Here, we assess the treatment-potential of antisense, splice-correcting oligonucleotides (SCOs) targeting mutated BTK transcripts. Both the SCO structural design and chemical properties were optimized using 2'-O-methyl, locked nucleic acid (LNA) or phosphorodiamidate morpholino (PMO) backbones. In order to have access to an animal model, we engineered a novel, Bacterial Artificial Chromosome (BAC)-transgenic mouse carrying an authentic, mutated, splice-defective human BTK gene. To avoid interference of the orthologous mouse protein, mice were bred onto a Btk knockout background. This represents a unique model to study treatment strategies for diseases resulting from splice-site mutations. For the first time the capacity of SCOs to correct aberrantly spliced BTK in B lymphocytes, including pro-B cells, is demonstrated. The corrected BTK mRNA restored expression of functional protein as shown both by enhanced lymphocyte survival and re-established BTK activation upon B cell receptor stimulation. As final proof-of-concept BTK restoration *in vivo* in mice and *ex vivo* in primary patient cells was obtained. Thus, our approach may represent a future personalized medicine for XLA.

Keywords: Primary immunodeficiency; B cell; pre-mRNA splicing; oligonucleotide therapeutics; mouse model.

[S-0120]

[Inflammation and Diseases]

A new humanized mouse model for autoimmune cardiomyopathy and its use to devise immunomodulation therapy

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Myocarditis is the principal cause of heart failure in young adults. Frequently triggered acutely by an episode of viral infection, its progression to dilated cardiomyopathy is associated with the development of auto-immunity, especially to human cardiac α-myosin (hCAM). Consistent with this, HLA genotype influences prevalence of the disease. Previous studies showed that humanised DQ8 transgenic non-obese diabetic mice spontaneously developed autoimmune cardiomyopathy, whereas the DR4 allele is over represented in patients and there is no association with diabetes. We therefore attempted to induce experimental autoimmune myocarditis in DR4 transgenic mice (DR4 mice) as a more relevant model of the human disease.

DR4 mice were injected with purified hCAM or vehicle subcutaneously in complete Freund's adjuvant (CFA). After 3 weeks, anesthetized mice were subjected to cardiac ultrasonography, following which blood was obtained from the abdominal aorta under terminal anaesthesia. The hearts were then perfused fixed for histology and spleens were harvested for proliferation assay. Potential immunomodulatory peptides were predicted in silico. Peptides were then proven water soluble and effective in T-cell proliferation assays. For immunotherapy, mice were pre-dosed with escalating doses of mixtures of 3 each of 6 soluble hCAM-derived peptides (pools 1 and 2) according to an established protocol.

DR4 mice did not develop spontaneous myocarditis. However, all mice immunized with hCAM developed high titres of both IgG1 and IgG2c antibodies. Consistent with this, splenic T-cell proliferation responses to hCAM significantly increased compared to un-immunized mice. DR4 mice immunized with hCAM failed to gain weight and by echocardiography showed a significant decline in cardiac output and fractional shortening and increase in diastolic dimension compared to those injected with PBS in CFA alone. 5/5 immunized vs 0/5 control mice showed cardiac inflammation based on histology. 3/5 immunized mice died if the experiment was prolonged for 6 weeks. Pre-treatment with hCAM derived peptide pools 1 or 2 blunted the T-cell proliferation response and pool 2 also decreased both IgG1 and IgG2c levels. Pools 1 and 2 significantly improved the left ventricular cardiac function by increasing the percentage of ejection fraction and fractional shortening. Pool 2 also significantly reduced cardiac inflammation.

We have developed a novel, more relevant humanized mouse model of autoimmune cardiomyopathy and demonstrated its ability to validate the immunomodulatory activity of hCAM derived peptides. Further use of our approach should prove valuable in developing optimized, clinically applicable peptide cocktails to prevent the progression of myocarditis to dilated cardiomyopathy.

Keywords: Autoimmunity; inflammation; immunotherapy; myocarditis; cardiac myosin.

[S-0134]

[Tumor Immunotherapy]

Encapsulation of TLR9 and iNKT cell ligands into exosomes induces immune responses and prevents tumor development

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Exosomes are natural nano-vesicles secreted from all types of cells. Due to their important biological functions, utilization of exosomes as delivery system became the central interest of exosome research. Invariant natural killer T (iNKT) cells are a unique subset of T cells. They have important biological functions modulating both innate and adaptive immunity in response to self and bacterial lipid antigens. Alpha-galactosylceramide (α-GalCer) is a widely used glycolipid ligand for iNKT cells. In this study, we aimed to investigate the degree of immune activation of CpG ODN and α-GalCer encapsulation in the presence or absence of cancer antigen ovalbumin (OVA) within exosomes and subsequent studied the ability of these constructs on the control of tumor development in mice.

Exosomes were isolated from mouse macrophage cell line RAW264.7 by differential ultracentrifugation and encapsulated with Ovalbumin (OVA), CpG ODN and α-GalCer by lyophilization method. Exosome quality were characterized by a bead-based flow cytometry method. ELISA and Western Blot were used to determine the amount of OVA association with the exosomes. To assess the immunological responses, mice were injected at day 0 intraperitoneally with exosomes loaded with different combinations of free and exosomal CpG ODN/antigen/α-GalCer. Mice were fed with 5-bromo-20- deoxyuridine (BrdU) in drinking water for 7 days. At day 7, blood and splenocytes were collected. Production of IFNγ, ova-specific serum IgG and cell-specific surface staining were done by ELISPOT, ELISA and flow cytometry, respectively. Noext, therapetic effectiveness of the exosomal constructs were tested on mice that were subcutaneously inoculated with B16-OVA melanoma cells ($1x10^6$/mice). On days 9 and 15, exosomal therapies were performed on tumor bearing mice and tumor size reduction was followed.

Flow cytometry analyses revealed that exosomes retained classical surface markers even after reconstitution method. ELISA and Western analyses demonstrated that majority of OVA was encapsulated within exosomes. Cytokine induction ability was significantly pronounced when α-GalCer was included within exosomes, and further augmented when CpG ODN was co-encapsulated within exosomes. *In vivo* tumor challenge studies with C57/Bl6 mice indicated that tumor mass reduction was significantly higher with α-GalCer containing exosomes than exosomes without α-GalCer.

This study demontrated that co-encapsulation of TLR9 and iNKT ligands within exosomes together with cancer antigen is more potent inducing both innate and adaptive immune responses thereby help to control established tumors in a therapeutic cancer vaccine setting.

Keywords: Exosomes; CpG ODN; iNKT cells; alpha-galactosylceramide; cancer; immunotherapy.

[S-0161]

[Immunodeficiencies: Emerging Molecular & Cellular Therapies. Remembering Prof. Isıl BARLAN]

Immunodeficiency and immune dysregulation in DOCK8 deficiency: clinical and mechanistic studies in a defined patient cohort

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DOCK8 is G protein exchange factor (GEF) that regulates the actin cytoskeleton by virtue of its activation of the small G protein CDC42.DOCK8 deficiency is a combined immune deficiency characterized by recurrent infections, eczema, food allergy, increased serum IgE levels and low T cell numbers.We have identified sixty patients with DOCK8 deficiency from 53 different families, including 41 patients from Turkey. Thirty-nine distinct mutations were found in this cohort. Forty-one patients (69.4%) had insertions or deletions, and seventeen had homozygous point mutations, including nonsense (10/11) or splice site mutations (7/17). Only one patient was found to have a true missense mutation that abrogated the GEF activity, indicating that failure of CDC42 activation by DOCK8 is necessary and sufficient for disease development. Analysis revealed certain mutant DOCK8 alleles that are prevalent in different areas in Turkey including deletion of exon 1-27, found in patients from western Turkey (Izmir; n=6), and a splice donor site mutation in intron 16 common in central Turkey (Konya; n=5). All the described mutations and deletions resulted in loss of protein expression, except for the one missense mutation.

Comprehensive analysis of our cohort, followed by us since 2006, revealed recurrent infections (90.9%), eczema (88.4%), viral skin infection (58.6%), food allergy and asthma (42.8%) as the most common clinical features of the disease. Six patients had malignancy, four of them over 9 years of age. Five patients with autoimmune disease did not have any sign of autoimmunity at the beginning. They had autoimmune diseases after 4 years of age. Six patients were diagnosed under 2 years of age because of family history and/or in the course of allergic evaluation. These patients had no history of recurrent infections. The youngest of the six, aged six months, had normal IgE and no allergic manifestations or eczema. Thus disease manifestations maybe missed early in life.

Evaluation of Th2 cytokines by flow-cytometry revealed increased IL-4 production, consistent with Th2 skewing and clinical allergic manifestations. Circulating T regulatory (Treg) cells and T follicular regulatory (TFR) cell frequencies were decreased when compared to controls. In contrast, circulating TFH cell frequencies were significantly higher in patients, consistent with their propensity to develop autoimmunity. Evaluation of autoantibody production by autoantigen microarray screening showed significantly increased antibody production. In summary, DOCK8 deficiency is an immune dysregulatory disease that presents with immune deficiency, allergic diathesis and autoimmunity and viral-driven malignancies. Further analysis of the mechanisms of immune dysregulation, especially as relates to Treg and TFH cell abnormalities, may be particularly relevant to managing allergic and autoimmune complications of this disease.

Keywords: Allergy; DOCK8 deficiency; immune dysregulation; T regulatory cells; T folicular cells; T folicular regulatory cells.

[S-0162]

[Regulatory Cells of Immune System]

The effect of activated allogeneic macrophages on tumor immunity in experimental lung cancer

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Introduction and Aim: Even though macrophages are the primary APCs located in the tumor microenvironment, these cells become dysregulated and gain an immune suppressive character supporting tumor development. In this study, modulation of immune responses upon administration of LPS-activated allogeneic macrophages into lung tumor-bearing hosts was evaluated.

Methods: Following the establishment of Lewis lung carcinoma (LLC-1) tumors, the C57BL/6 mice were injected with LPS-stimulated allogeneic J774.A1 macrophages. J774 cells were subcutaneously injected into a distant (peripheral injection, PF-group) or a peri-tumoral site (PT-group). One group received peripheral injection of macrophages 24h after peri-tumoral administration of the cells (PT+PF group). PBS injections were used as mock treatments and a control group received only PBS. The change in tumor size and number of metastases were determined by macroscopic and histopathological evaluation. Activation and distribution of T cells found in the tumor and in the spleen were assessed with flow cytometric analysis of CD4, CD8, CD25, CCR5 and IFN-γ. *In vivo* distribution of administered J774 macrophages determined with eFluor670 staining. Expression of proinflammatory cytokines TNF-α, IL-12p40, IL-1β and IFN-γ in tumor and spleen tissues was evaluated with RT-PCR.

Results: Peri-tumorally administered macrophages were detected in the tumors confirming their infiltration into the microenvironment. Growth of lung tumors was significantly reduced especially in the group that received the J774 peripherally (PF-group). The mice in this group were free of metastases whereas other groups displayed high incidence of metastatic foci in the lung. The spleen of PF-group mice were highly infiltrated by CD4+ helper and CD8+ cytotoxic T cells. The levels of CD4+CD25+ cells in tumor or spleen were not differed amongst the groups. Compared to the control tumors, CD4+CCR5+ were decreased following J774 injection in all groups. IFN-γ mRNA was hardly detected all groups. IL-12 and TNF-α were higher in the tumor and spleen samples obtained from the PF-group. Splenic IL-1β gene expression was significantly increased in all groups administered with J774 cells. Interestingly, TNF-α and IL-12 were decreased in the tumor whereas increased prominently in the spleen of PT+PF mice.

Conclusion: These results provide information on the efficacy of non-specific allogeneic immune responses triggered in the sites distant from primary tumors. In addition, this study reinforces our knowledge on the immune regulation/suppression in the tumor microenvironment.

This study is supported by Hacettepe University Scientific Research Projects Coordination Unit (Project no: 014 A104 001-346).

Keywords: Lung cancer; macrophage; allogeneic reaction; tumor immunity.

[S-0163]

[Novel Discoveries in the Innate Immune System]

APPL1 involvement in TLR3/4-dependent TBK1 and IKKε activation in macrophages

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Endosomes have important roles in intracellular signal transduction as a sorting platform. Signaling cascades from TLR engagement to IRF3-dependent gene transcription rely on endosomes, yet the proteins that specifically recruit IRF3-activating molecules to them are poorly defined. We show that adaptor protein containing a pleckstrin-homology domain, a phosphotyrosine-binding domain, and a leucine zipper motif (APPL)1, an early endosomal protein, is required for both TRIF- and retinoic acid-inducible gene 1-dependent signaling cascades to induce IRF3 activation. APPL1, but not early endosome Ag 1, deficiency impairs IRF3 target gene expression upon engagement of both TLR3 and TLR4 pathways, as well as in H1N1-infected macrophages. The IRF3-phosphorylating kinases TBK1 and IKKε are recruited to APPL1 endosomes in LPS-stimulated macrophages. Interestingly, APPL1 undergoes proteasome-mediated degradation through ERK1/2 to turn off signaling. APPL1 degradation is blocked when signaling through the endosome is inhibited by chloroquine or dynasore. Therefore, APPL1 endosomes are critical for IRF3-dependent gene expression in response to some viral and bacterial infections in macrophages. Those signaling pathways involve the signal-induced degradation of APPL1 to prevent aberrant IRF3-dependent gene expression linked to immune diseases.

Keywords: APPL1; TBK1; IKKepsilon; TLR3/4; innate immunity.

[S-0168]

[Cellular and Molecular Targets in Rheumatoid Arthritis and Autoimmune/Autoinflammatory Diseases]

Characterization of NK cells in patients with multiple sclerosis under sphingosine-1-phosphate receptor 1 (S1P1) antagonist therapy

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Introduction and Aim: Multiple sclerosis (MS) is a chronic inflammatory disease of central nervous system. The role of natural killer (NK) cells in immune pathogenesis of MS is becoming more evident now that the use of immune modulatory drugs are involved. This study focuses on the characterization of NK cells in a group of MS patients receiving fingolimod, a functional antagonist of sphingosine-1-phosphate receptor 1 (S1P1) which interferes with the T lymphocyte egress from the lymph nodes. This study aims to investigate the status of NK cells upon fingolimod-mediated the reduction of circulating T lymphocytes.

Methods: Peripheral blood samples were collected from patients with Relapsing Remitting MS (RRMS) under immune modulatory drugs other than fingolimod (n=23), and RRMS patients under fingolimod treatment [patients were followed up for 1 month (n=15), for 3-6 months (n=16), and for 12-24 months (n=15)]. Age- and sex-matched healthy blood donors were used as control group. Peripheral blood mononuclear cells (PBMCs) were isolated and expression of T and NK cell markers (CD3, CD16, CD56, CCR7, NKG2D, NKp46, IFN-gamma and CD107a) were analyzed with multi-color flow cytometry. Additionally, PBMCs were co-cultured with K562 cell line which spontaneously activates NK cells. Then, IFN-gamma production and degranulation capacity of NK cells were assessed.

Results: Expectedly, lymphocyte counts were dropped drastically in the early phase of S1P1 antagonist treatment (≤6 months). Loss of CCR7 expression on the remaining T cells was also determined. Due to the decrease in T lymphocytes (CD3+CD16/56-), the percentage of NK cells (CD3-CD16/56+) was found to be upregulated whereas the numbers of these cells were the least affected from lymphopenia. Notably, especially in the later periods of the treatment (≥6 months) the amount of CD56brCD16+/-subset of NK cells were decreased while the proportion of CD56dimCD16+ subset were significantly upregulated. Accordingly, these cells were also determined with higher expression of NKG2D and NKp46 activating receptors. Degranulation capacity of NK cells (CD107a expression) was enhanced especially after 12 months of the treatment whereas IFN-gamma production was not affected.

Conclusion: Even though the number of NK cells was also affected by treatment with S1P1 antagonist, especially the percentage of CD56dim subset was increased and displayed potent cytotoxicity and did not lose capacity to produce IFN-gamma. Our results indicate a compensatory role for NK cells in the absence of T lymphocytes in RRMS patients under fingolimod therapy.

Keywords: Multiple sclerosis (MS); natural killer (NK) cells; sphingosine-1-phosphate receptor 1 (S1P1) antagonist; fingolimod therapy.

[S-0174]

[Novel Discoveries in the Innate Immune System]

Molecular mechanisms for adenosine regulation of dendritic cells

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Tissue extracellular adenosine concentrations and expression of G protein-coupled adenosine receptors are elevated after cell death, injury and inflammation. Recently we, and the others, have shown that dendritic cells as one of the primary targets for adenosine to suppress T and NK cell responses within the tumor microenvironment. However, molecular mechanisms for adenosine regulation of dendritic cells are poorly understood. Here we show that inflammatory LPS strongly elevated adenosine A2 subtypes of adenosine receptors in dendritic cells. By using stable adenosine analog NECA we show that adenosine receptor signaling increased LPS-induced anti-inflammatory IL-10 while suppressing effector cytokines for Th1 polarization such as IL-12 and TNFalpha. Adenosine also significantly reduced cell surface expression of MHCI and CD86. Adenosine did not affect phospho-activation of inflammatory signaling pathways such as NFkappaB and IRF3. A2 adenosine receptor stimulation can potentially increase MAPK activation and cAMP levels to regulate cellular responses. Although we did not observe increased MAPK phospho-activation we did observe that cAMP analogs specifically targeting EPAC or PKA pathways phenocopied adenosine's effects on dendritic cells especially when used together. Adenosine-mediated suppression of inflammatory/effector cytokines and increase in pro-inflammatory IL-10 is associated with increased expression of anti-inflammatory c-Fos and NR4a receptors, which are known to be targeted by cAMP and suppress NFkappaB to reduce inflammatory responses. Overall our results suggest that adenosine targets both cAMP/EPAC and cAMP/PKA pathways and increase the expression of c-Fos and NR4A to modulate dendritic cell responses. Our findings have important implications for the development of novel therapies for immune related diseases such as cancer by targeting adenosine receptors. (This work is supported by grants from The Scientific and Technological Research Council of Turkey-TUBITAK- project no: 214S659, 115C062 and 115Z170)

Keywords: Adenosine signaling; cAMP signaling; immunoregulation.

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[Inflammation and Diseases]

Presentation of two AQP4 IgG seronegative NMOSD diagnosed patients according to 2014 IPND criteria

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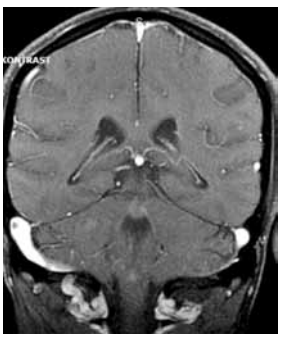
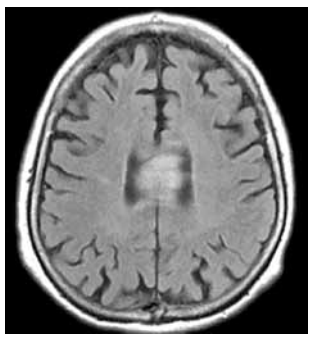
Since the proposal of new diagnostic criterias by The International Panel for NMO Diagnosis, NMOSD umbrella term gave clinicians the opportunity to catch more patients with this syndrome. Since the differation between MS and NMOSD is essential for treatment, new criterias warrant more accurate and early diagnoses and leave an open door for future arrangements. We would like to share the interesting presentations of two AQP4 IgG seronegative NMOSD cases to demonstrate NMOSD suggesting radiological properties. 87 years old women presented with long extended transverse myelitis and had three more attacks in 17 months, medulla obongata involvement, optic neuritis and transverse myelitis with optic neuritis, respectively. Corpus callosum involvement was observed in repeated gadolinium enhanced MRI. 53 years old man presented with area postrema syndrome and diencephalic involvement. Repeated gadolinium enhanced MRI revealed long extended transverse myelitis, optic chiasma and posterior optic tract involvement and vast ependimal gadolinium enhancement in fourth ventricle, central channel and medulla spinalis.

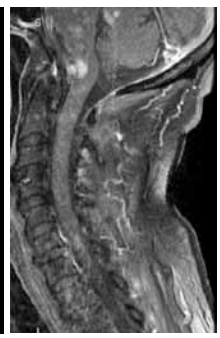
Keywords: AQP4 IgG seronegative NMOSD, corpus callosum involvement, diencephalic involvement, area postrema syndrome.



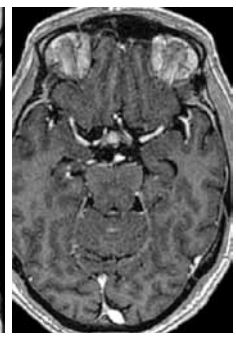
Gd+ 4th ventricle and dorsal medulla lesions case 2.



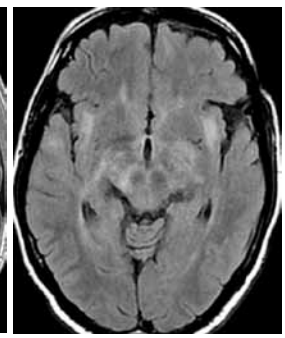
Gd+ C Callosum lesion case 1.



Gd+ Medulla and spinal cord lesions case 2.



Gd+ Opric chiasma and posterior tract lesions case 2.



T_2 Weighted image white matter lesion case 2.

[P-0011]

[Immunity to Infection: New Trends and Developments]

Expression levels of inflammasome genes and Caspase-1 during acute brucellosis

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Brucella spp. are gram-negative, facultative intracellular bacteria that cause acute and chronic zoonotic disease. The consumption of contaminated foods, especially unpasteurized milk and dairy products, and more rarely occupational exposures can result in infection in humans. It not only is a huge economic burden in developing countries, but it is also a potential bio-terrorism agent in the United States since it is a class III pathogen. The clinical forms of human brucellosis are determined according to the duration of symptoms and are described as acute (less than 8 weeks), subacute (from 8 to 52 weeks) and chronic (more than 1 year). The immune response varies according to the clinical forms. In acute brucellosis, overproduction of T helper-1 (Th1) cytokines, primarily interferon-gamma (IFN-γ) and IL-2, has been detected in serum samples and in the intra-cytoplasmic niche. Adequate antibiotic treatment reduces or normalizes these levels.

As known; pathogens are detected by innate immune receptors that, upon activation, orchestrate an appropriate immune response. Recent studies revealed that intracellular signaling cascades involved in the TLR-initiated immune response to Brucella infection. However; no report was elucidated the role of inflammasome receptors expressions in Brucellosis. Inflammasomes have crucial roles on host to perform appropriate innate immune response. They act either by inducing pro-inflammatory cytokine IL-1β and IL-18 secretion or by inducing pyroptosis on infected cells. In both of them Caspase 1 activation is essential. First time in our investigation we observed the expression level of Caspase 1 and the inflammasomes AIM2, NLRC4, NLRP3 that role in to activate Caspase 1 during acute brucellosis.

Peripheral blood samples were obtained from 19 acute brucellosis patients and 19 healthy controls. RNA isolation and following that cDNA synthesis were all performed from peripheral blood samples. In our results we observed significant elevation on AIM2 and NLRC4 expression in acute brucellosis patients in comparison to healthy controls. Although AIM2 and NLRC4 are the inflammasomes responsible from Caspase 1 activation we did not observe a significant elevation on acute brucellosis patients in comparison to healthy control.

As a result AIM2 and NLRC4 expression occurs during acute brucellosis however those genes expressions are not enough to activate Caspase 1 expression. That is probably one of escape mechanism of bacteria from pyroptosis in order to replicate in its niche.

Keywords: AIM2; NLRC4; NLRP3; Caspase 1; Acute Brucellosis.

[P-0014]

[Novel Discoveries in the Innate Immune System]

A DNA aptamer binds to hCD3+ T-lymphocytes and granulocytes but does not bind to hCD19+ B-lymphocytes

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Aptamers are nucleic acid oligomers with distinct conformational shapes that allow binding targets with high affinity and specificity. Selective evolution of ligands by exponential enrichment (SELEX), an *in vitro* selection process to develop aptamers, ha been invented in 1990. Aptamers are not only used in therapeutic applications but are also talent in diagnostic applications.

In our laboratory we invented an aptamer DNA binds to human CD3+ lymphocytes and granulocytes but does not bind to human CD19+ B-lymphocytes by using CELLEX technology. We targeted primers to a conserved sequence at human CD3ε chain that is also used to synthesize monoclonal antibodies against CD3ε chain. We synthesized a nucleotide library containing a random segment of N(44) nucleotides flanked by 5'-ACC TCG TTC TTA TCT- and -G TCC GTT TCC CCA TT-3' primers by hybridizing them with CD3+ T-lymphocytes obtained from peripheral blood sample of an healthy individual (Figure 1). After 4 cycle hybridizing and selection processes, we obtained 4 different aptamers and randomly one of them named as A-CD3-1 was analyzed to observe binding capacity to mononuclear cells.

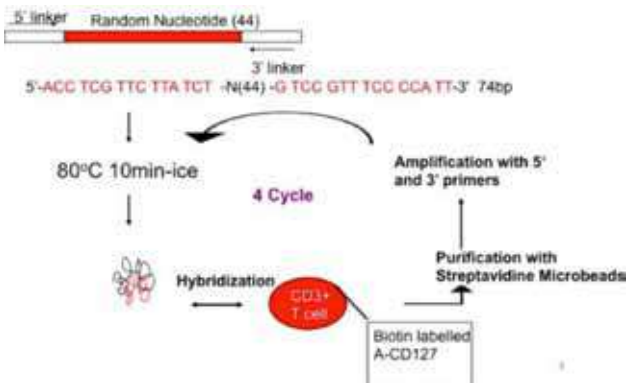

Figure 1.

According to our flow cytometry experiments, we observed that A-CD3-1 binds to granulocytes and CD3+ T-lymphocytes. However, it does not bind to CD19+ B-lymphocytes. It keeps its binding capacity till to 5nmole concentration. Activation index studies and potential use of A-CD3-1 aptamer in B-lymphocyte isolation are still under investigation. In order to increase specificity of aptamers either increasing the cycle of CELLEX procedure or using a peptide molecule related with conserved sequence of CD3ε chain will be useful.

Keywords: Aptamer; hCD3; hCD19.

[P-0022]

[Inflammation and Diseases]

Interleukin 10 level is correlated with SOFA score but not procalcitonine during sepsis

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Introduction: New findings suggest that there are two distinguished stage of sepsis: early one is dominated by an inflammatory profile of cytokine and the late one by an anti-inflammatory profile of cytokine with mainly interleukin 10 (IL-10). The level of IL-10 has been suggested to be related with clinical worsening during sepsis. Our aim was to study the correlation between the level of IL-10 and SOFA score during sepsis.

Methods: Septic patients were enrolled in a prospective descriptive study. Blood samples were collected at three times: hour 0 (H0), H24 and H48. H0 was the Diagnosis of sepsis time. Serum levels of IL-10, TNF and IL-6 were measured with the technique of a solid-phase, two-site chemiluminescence enzyme immunometric assay (Immulite 1000, Siemens, USA). Procalcitonin (PCT) was measured at H0, H24 and H48. SPSS 11.0 was used. Friedman test was used to compare nonparametric variables. It is statistically significant if $p<0.05$. Correlations between variables were determined by using the Pearson correlation analysis for normally distributed variables. Quantitative variables are expressed as median (Interquartile range).

Results: Twenty six patients were included. There was an elevation of IL-6 and IL-10 during the first 24h. The mean value of IL-6 at H24 was higher than H0: respectively 114.99 pg/ml [10.32-50.35] and 117.13 [21.7-118.2] but not statistically significant ($p=0.6$). The mean value of IL-10 at H24 was higher than H0 respectively: 30.75 [5-21.85] and 59.28 [13.8-39] but not statistically significant ($p=0.11$). At 48 hours, IL-6 and IL-10 were decreased respectively 22.9 [15.8-28.4] and 8.76 [4.35-12.55]. P was not significant. There was no significant change of TNF level at different times. PCT levels at H0, H24 and H48 were respectively: 2.1 [0.2-4.1], 0.7 [0.24-0.7] and 6.16 [0.05-14.9]. P was not significant ($p=0.3$). There was a correlation between the level of IL-10 and SOFA score at H0 ($R=0.8/p=0.039$) but it wasn't a correlation between TNF, IL-6 and proclacitonine with SOFA score.

Conclusions: Level of IL-10, mainly at H0, is correlated with prognosis during sepsis. Therefore, these cytokines might be a new tools for management of this pathology. However, further studies are needed to confirm our results.

Keywords: Sepsis; IL-10; cytokine and SOFA score.

[P-0025]

[Regulatory Cells of Immune System]

Immune complexes regulate Th17 responses through coordinated suppression of IL1 and enhanced IL10 production by dendritic cells

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The exact mechanism in which the innate immunity is stimulated is crucial for shaping the following CD4+ T helper (Th) responses. The antigen downregulates Th1 responses when encountered in the presence of antigen-IgG immune complexes. To evaluate if Th17 responses are regulated in a similar manner in the presence of antigen-IgG immune complexes, we used an inflammatory airway disease model by immunizing mice with Complete Freund's Adjuvant (CFA) and ovalbumin (Ova) that induces a powerful Ova-specific Th1 and Th17 responses. When the immunization with CFA-ova was modified with the addition of ova specific IgG (CFA-Ova-IgG), Th17 responses were suppressed and ova-specific Th2 responses were enhanced. Moreover, we demonstrated the mechanism by these immune complexes diminish Th17 responses is via the suppression of dendritic cell IL-1 production and the elevation of IL-10 production. Together these data depict a novel mechanism by which the Th17 responses are regulated.

Keywords: Immune complex; inflammasome complex; Th17 response.

[P-0026]

[Inflammation and Diseases]

Pharmaco-modulation of splenic inflammatory response by histamine receptors during scorpion envenomation pathogenesis

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The inflammatory response is a key process in the genesis of the pathological events during scorpion envenomation. The activation and release of pro-inflammatory mediators such as cytokines and vasoactive substances may play an important role in the pathophysiology of scorpion envenomation.

The spleen organ is one of the tissues that could be damaged after scorpion envenomation. The mechanism of the induced splenic inflammation by scorpion venom has not yet been elucidated and was little investigated. The present study aimed to evaluate effects of scorpion venom on this lymphoid organ and to investigate the potential involvement of histamine receptors in the induced splenic inflammatory response by Androctonus australis hector (Aah) scorpion venom. Indeed, selective histamine receptor antagonists (cetirizine, cimetidine, betaserc and JNJ-7777120) were administered before the envenomation of mice.

The inflammatory response was evaluated by assessing vascular permeability changes and inflammatory cell recruitment including polymorphonuclear and eosinophil cells into this organ. On the other hand, splenic disorders were evaluated by the measurement of some markers of oxidative/nitrosative stress such as nitric oxide, malondialdehyde and reduced glutathione but also by histopahological analysis.

The results showed significant alterations of the splenic tissue marked by an increase of vascular permeability and inflammatory cells recruitment, as well as an increased nitric oxide levels and splenic membranes lipids peroxidation concomitant with reduced antioxidant defense.

The histamine H1 and H4 receptor antagonists (cetirizine and JNJ7777120, respectively) seem to be more efficient in the prevention of the induced splenic disorders by scorpion venom than the H2 and the H3 receptor inhibitors (cimetidine and betaserc, respectively). A significant reduction of the vascular permeability, the inflammatory cell infiltration and a marked prevention of oxidative/ nitrosative stress markers were observed. Our results suggest the involvement of histamine receptors in the induced splenic inflammatory response by scorpion venom, especially the H1 and the H4 receptors but suggest also the usefulness of their inhibitors in the amelioration of the therapeutic management of scorpion envenomation cases.

Keywords: scorpion envenomation; splenic tissue; inflammatory response; oxidative stress; histamine receptors.

[P-0027]

[Inflammation and Diseases]

The amplifying role of the inflammatory response in the pathogenesis of skin tissue damage induced by (Cerastes cerastes) venom

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Viperidae venoms cause a prominent local tissue damage associated with a multifactorial and complex immune response. Snake Venom Metalloproteinases (SVMPs) are mainly involved in the induced local pathological alterations and play a relevant role in the induced inflammatory response characterized by an infiltration of leukocytes and the release of endogenous mediators that trigger local inflammatory disorders leading to tissue damage. In this study, we analyzed the implication of local inflammatory response in the development of skin tissue damage after (Cerastes cerastes) envenomation. Obtained results revealed that the venom induced a strong dermonecrotic activity after 72 hours of envenomation associated with an intense inflammatory reaction marked by a significant increase of IL-6 and TNF-α levels. Zymography analysis of skin homogenates showed that SVMPs were involved in the release of MMP-9 and also in the activation of MMP-2, playing thus a relevant role in the pathogenesis of tissue damage. The inflammatory exudate collected after envenomation showed a conspicuous release of MMP-9 and MMP-2. The co-injection of the exudate with the venom significantly amplified tissue damage. Venom induced an increase of myeloperoxydase activity and NO levels. The co-injection of the venom with the inflammatory exudate induced an increased NO levels. EDTA treatment of the venom significantly reduced myeloperoxidase activity and NO levels in tissue homogenates.

These results suggest that SVMPs play a relevant role in the induced tissue damage by a direct effect owing to their proteolytic activity on extracellular matrix and basement membrane and also by an indirect effect involving a complex inflammatory process inducing the release of several endogenous mediators including MMPs. The comprehension of the inflammatory mechanisms underlying tissue damage will lead to interesting therapeutic strategies in the treatment of viper envenomation.

Keywords: Venom; (Cerastes cerastes); tissue necrosis; inflammation; exudate; metalloproteinases.

[P-0033]

[Tumor Immunology & Molecular Markers]

Can IL-25 and IL-17B promote the malignancy of ER positive and ER negative breast cancer cell lines?; an *in vitro* evaluation of IL25 effects versus IL-17B on breast cancer cell lines

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Introduction: Cytokines are one of the most important tumor microenvironment components. IL-25 (IL-17E) and IL-17B are two members of IL-17 family, which bind to a unique receptor (IL-17RB). IL-17RB is widely expressed on breast cancer tissue and IL-25 apoptotic effect is not established. Since tumor surrender non-malignant cells produce IL-25, the role and competition of IL-25 and IL-17B might be important in tumor fate. We investigated if tumors growth could be controlled spontaneously by apoptotic effect of IL-25, why overexpressing of IL-25R would be observed in poor prognosis breast cancer cell lines as well as possible antagonistic effect of IL-25 and IL-17B in breast cancer cell lines.

Materials-Method: MCF-7 (Estrogen receptor positive) and MDA-MB231 (Estrogen receptor negative) breast cancer cell lines and MCF-10A as a non-malignant cell line was treated with human recombinant cytokines (rhIL-25/IL-17E and rhIL-17B). Apoptosis test was performed using Annexin V and PI staining and analyzed by flow cytometery. Proliferation rate was evaluated by MTT assay. Gene expression analysis was performed for IL-25R (IL-17RB), IL-17B and MMP-1 (Matrix-Metalo Proteinase) by Real Time PCR before and after cytokine treatment in malignant and non-malignant cell lines.

Results: The results showed that IL-25 treatment induces apoptosis up to 20% and IL-17B promotes the cell viability percentage in malignant cell lines without any apoptotic or proliferative effect on non-malignant cells. In combinational cytokines treatment the apoptosis assay showed antagonistic effect of IL-17B. The gene expression analysis results showed IL-25R in ER+ cell lines, is overexpressed in responses to both cytokines. IL-17B induced overexpression of IL-17B mRNA level in IL-17B+ cell lines (MCF7, MDA-MB231) and IL-17B negative cell line (MCF-10A). Both cytokines induced elevated level of MMP-1 expression in invasive cells (MDA-MB231).

Conclusion: IL-25 increased the malignant cell lines apoptosis without toxicity on non-malignant cell lines and IL-17B increase cell viability without effect on non-malignant cells. Non-malignant cells produce IL-25 and gene expression analysis following treatment by IL-25 showed increased IL-17B and IL-25R mRNA expression level which possibly leads to MCF-7 cell survival and MDA-MB231 invasiveness. The over expression of IL-17B in IL-17B negative cells was very important point in tumor and non-malignant cells micro-environment alteration in response to IL-17B which elevated in malignant cells in response to IL-25. ER+ cells malignancy in response to IL-25 and IL-17B alter by IL-25R expression and ER negative cells malignancy elevated by IL-17B effects on MMP expression.

Keywords: IL-17B; IL-25; IL-17RB; MCF-7; MDA-MB231; MCF-10A.

[P-0034]

[Emerging Issues in Adaptive Immune System]

Altered peptide ligands: qualitative or quantitative effects on signaling in monoclonal T cells?

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Objectives: Altered peptide ligands (APLs) are derivatives of antigenic peptides containing substitutions at residues involved in T cell receptor (TCR) contact or major histocompatibility complex (MHC) binding. While these substitutions can quantitatively affect T cell signaling following APL stimulation, the question remains whether APLs can also have qualitatively different TCR signaling effects.

Materials-Methods: In this study, we employed the Marilyn (CD4+ anti-H-Y) TCR transgenic system and designed several H-Y-derived APLs bearing substitutions at MHC-II anchor or TCR contact residues. Marilyn PD-1–/–T cells lacking a co-inhibitor and thus thought to be biased toward enhanced TCR signaling were also included in the assays to examine threshold effects that could mediate qualitatively different responses to APLs. The proliferative response to different concentrations of native H-Y or these APLs was examined by both *in vivo* and *in vitro* proliferation assays using mixed Marilyn and Marilyn PD-1–/–T cells. The ability of the APLs to bind MHC-II and potential for particular APLs unable to stimulate proliferation to inhibit T cell responses to native H-Y peptide were also assessed.

Results: We found that Marilyn and Marilyn PD-1–/–T cells proliferated similarly in response to H-Y and all of the APLs tested in a dose dependent manner and in accordance with their predicted TCR and MHC affinity. Particular APLs could block the proliferative response of T cells to H-Y in co-culture.

Conclusions: Overall our data suggest that APLs have primarily quantitatively different signaling effects on T cells, although some APLs may have utility in blocking the response to native epitopes. Our research indicated that novel further studies should be carried out to evaluate the mechanisms underlying the effects of APLs before research on APLs are translated into medicinal drugs.

Keywords: APLs; Marilyn; PD-1.

[P-0036]

[Inflammation and Diseases]

T cell chemokine receptors and cytokines in asthma

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T cell subgroups are known to play important roles in the pathogenesis of allergic and non-allergic asthma. In this study the expression of the surface markers including CD4, CD8, CCR3, CCR4, CXCR3 and intracytoplasmic cytokine (IFN-g, IL-4 and IL-10) profiles of peripheral blood mononuclear cells in stimulated (PMA/ionomicin) and unstimulated conditions were investigated by flow cytometry. Stimulated Th cells increased their $CXCR3^+$ IFN-g, $CXCR3^+$ IL-4, $CCR3^+$ IFN-g, $CCR3^+$ IL-4, $CCR3^+$ IL-10, $CCR4^+$ IL-10 expressions in allergic asthma; but $CXCR3^+$ IFN-g, $CXCR3^+$ IL-4, $CXCR3^+$ IL-10, $CCR3^+$ IFN-g, $CCR3^+$ IL-4 and $CCR3^+$ IL-10 expression in non-allergic asthma. Cytotoxic T cells in allergic asthma expressed higher $CCR3^+$ IFN-g, $CCR3^+$ IL-4, $CCR3^+$ IL-10, $CCR4^+$ IL-4, $CCR4^+$ IL-10 after stimulation. On the other hand $CD8^+$ $CXCR3^+$ IFN-g and $CD8^+$ $CXCR3^+$ IL-10 expressions increased in non-allergic asthma under the same stimulation.

In our study T cells tended to elevate their intracellular cytokine levels in all study groups under both conditions. Comparing patients with allergic and non-allergic asthma, $CCR3^+$ and $CCR4^+$ Type 2 helper T cells produced higher amounts of IL-10 compared to Type 1 $CD4^+$ $CXCR3^+$ cells in allergic asthma. $CD4^+$ $CCR4^+$ cells expressed higher IFN-g and IL-10 levels without any stimulation in patients with allergic and non-allergic asthma respectively.

In summary, our results indicate the role of T cells in the pathogenesis of allergic and non-allergic asthma, but also the complexity of the disease which can not be explained by using only the simple Th1/Th2 paradigma.

Keywords: Asthma; allergy; chemokine receptors; cytokine; Th1/Th2

[P-0042]

[Cellular and Molecular Targets in Rheumatoid Arthritis and Autoimmune/Autoinflammatory Diseases]

Investigation of liver autoantibodies in anticentromere antibody positive patients

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Background: Anticentromere antibody (ACA) is considered to be a serological marker specific to CREST (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactylia, and telangiectasia) syndrome. In addition to being present in CREST syndrome, ACA is also detected in the sera of patients with autoimmune liver disease. In the study, anti-soluble liver antigen\liver pancreas antigen (anti-SLA\LP), anti-liver cytosolic antigen 1 (anti-LC1), anti-liver kidney microsomal antigen 1 (anti-LKM1), and anti-mitochondrial antibody M2 (AMA-M2) were evaluated in the patients who were positive anticentromere antibody.

Material-Methods: A total of 39 patients who were positive anticentromere antibody were enrolled in this study undertaken in Izmir Katip Celebi University Ataturk Training and Research Hospital, Microbiology laboratory between January 2015 and September 2015. Anticentromere antibody and liver autoantibody positiveness were analyzed. Anticentromere antibody and liver autoantibodies were studied by indirect immunofluorescence method (IIF) and immunoblotting method (IB), respectively. The patients who have anticentromere antibody negative were used as control group.

Results: It was detected that 3 of 39 patients (7.6%) showed positivity in one liver autoantibodies. All of the positive liver autoantibodies were AMA-M2 antibodies. No statistically significant difference was found between ACA and autoimmune liver autoantibodies.

Conclusions: In this study we reported our experience to provide evidence for detection of various autoantibodies as potential diagnostic or prognostic tests. Further studies that contain a broad range of patients may contribute to the field.

Keywords: Anticentromere antibody; liver autoantibody; autoimmune disease.

[P-0043]

[Immunodeficiencies: Emerging Molecular & Cellular Therapies. Remembering Prof. Isıl BARLAN]

FOXP3 expressions in artemis deficient patients

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Autoimmune diseases can accompany with Primer Immune Deficiency (PID). Regulatory T (Tr) cells are very important to control auto-reactive cells and seem to be very important in the pathogenesis of autoimmunity in PID. FOXP3 (forkhead boxP3) protein plays an important role in the development and function of Tr cells. DCLRE1C (DNA cross-link repair 1C) gene codes Artemis nuclear protein which is important for the V(D)J recombination and DNA repairing. Some of our patients with Artemis deficiency have autoimmune disease and granuloma formation during follow up period. We have planned to investigate the FOXP3 expression in the patients with Artemis deficiency and compare with the controls.

We studied FOXP3 protein expressions on the $CD3^+$ $CD4^+$ T cells by lyse-wash introcytoplasmic staining in seven patients with Artemis deficiency and six healthy controls by four color flowcytometry (Becton Dickenson, FACS Calibur).

We determined the mean ratio of FOXP3 expression was 11.42% in the patients with Artemis deficiency and 24.2% in healthy controls in the $CD3^+$ $CD4^+$ T lymphocyte gate. There was no statistically significiant difference between two groups (p=0.051). But when we compared with patients and the controls in terms of CD3/CD4/FOXP3, CD3/FOXP3 and CD4/FOXP3, there were statistically significiant difference (p values respectively; 0.003, 0.007 and 0.048). Mean values of mean fluorescence intensity (MFI) were determined in controls and patients 231.17 and 87.49 respectively. These

values were found as significiant statistically ($p<0.05$). We determined that the FOXP3 protein expression was found to be lower than the controls and suggest that this could be related with immune dysregulation in Artemis deficiency.

Keywords: Artemis; FoxP3; immune dysregulation.

[P-0046]

[Novel Discoveries in the Innate Immune System]

Evaluation of the immunomodulatory effect of gold nanocages on macrophages *in vitro*

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Gold nanoparticles are special of interest in biomedical research due to their unique optical and chemical properties in addition to their excellent biocompatibility. Recently, gold nanocages (AuNCs), a special design with ultra thin porous walls and hollow interiors, have shown great potential in the fields of cancer diagnostics and treatment not only as a photothermal therapeutic agent but also contrast enhancement agent for both optical coherence tomography (OCT) and photoacoustic tomography (PAT). However, the previous reports on the immune effects of AuNCs are very limited. Thus, more assays should be conducted to investigate the impact of AuNCs on the immune system cells especially phagocytic macrophages. In this research, freshly synthesized and characterized Au nanocages were used. The uptake of AuNCs by macrophage cell line Raw 264.7 was studied by microscopic and ICP-MS techniques. For cytotoxicity studies, in addition to XTT assay, Xcelligence system was used for real-time and label-free monitoring of cell viability of macrophages treated with various doses of AuNCs. The dose-dependent effects of AuNCs on nitric oxide production and pro-inflammatory cytokine expression in macrophages were investigated by Griess and ELISA methods. Collectively, the results of this research provides the optimum AuNCs concentrations without toxicity or side-effects arising from inflammatory responses. Moreover, this study helps to understand the activation of innate immunity after exposure to AuNCs *in vitro*.

Keywords: Gold nanocages; macrophages; immunomodulation.

[P-0047]

[Novel Discoveries in the Innate Immune System]

Enhancing lentiviral gene delivery to human natural killer cells for applications in cancer immunotherapy

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Natural Killer (NK) cells are members of the lymphocytic lineage that potently kill tumors and infected cells. The potential of NK cells to be used in cancer immunotherapy continues to inspire research on various aspects, ranging from *ex vivo* activation/expansion to genetic modification for maximizing anti-tumor effect of NK cells.

Genetic modification can be carried out by using viral vectors however; NK cells have strikingly low transduction efficiency with viral vectors when compared to other immune cells. We have hypothesized that the anti-viral defense system in NK cells limits lentiviral gene delivery. Therefore we have looked for small molecule inhibitors that act in anti-viral defense pathways downstream of pattern recognition receptors (PRRs) and thus affect viral gene delivery and expression in human NK cells.

BX795, a small molecule inhibitor of TBK1/IKKε kinase complex that acts downstream of RIG-I, MDA-5 and TLR3, has shown a significant increase in lentiviral gene delivery when used during transduction of human NK-92 cell line as well as primary NK cells. Our results so far have shown that there is no defect in viral vector entry to NK cells; nonetheless there are problems in viral gene integration in the first four hours upon viral vector delivery where the role of BX795 is seen most prominently. The current approach to identify potential genes playing roles in anti-viral defense in NK cells includes the genome-wide CRISPR knock-out (GeCKo) library and RNA sequencing for better understanding the pathways most affected by viral vectors. Meanwhile, candidate genes are being investigated at protein and RNA level for identifying signal transduction events taking place upon viral vector entry, based on the knowledge of wild type virus infections in literature.

Small molecule inhibitors are quite useful tools in understanding pathways involved with innate anti-viral detection to increase the efficiency and to shed light on mechanisms behind lentiviral gene delivery, both in NK cells and other cell types, for promising future applications in gene therapy and immunotherapy.

Keywords: Natural killer cells; lentiviral gene delivery; gene therapy; immunotherapy.

[P-0049]

[Novel Discoveries in the Innate Immune System]

Microscopic analysis of aneuploidy induced by the mutation of the Ccdc124 gene

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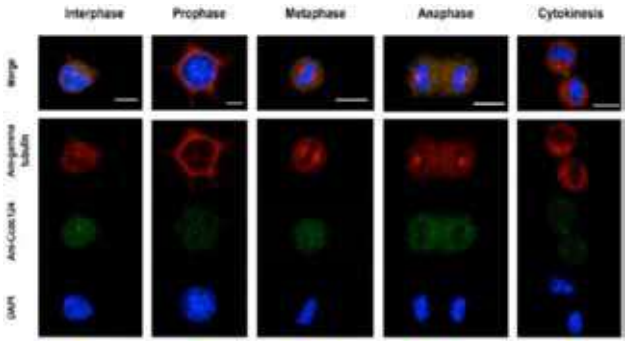
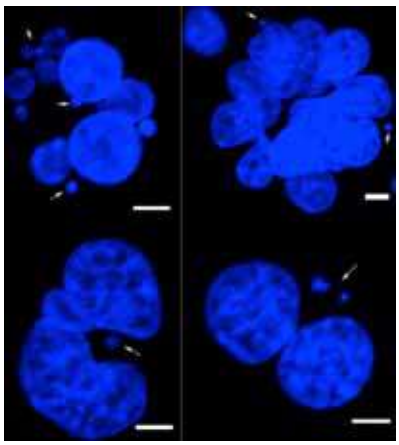
The Coiled-coil domain containing protein 124 (Ccdc124) is a centrosomal protein that relocates to the midbody region at the cytokinesis stage of the cell cycle. Cytokinetic abscission is the cellular process that leads to physical separation of two postmitotic sister cells by severing the intercellular bridge. Mutation of the Ccdc124 gene by CRISPR/Cas9 genome editing in HEK293T cells leads to the failure of cytokinesis and formation of aneuploid

(multinucleated-MN) aberrant cells. In this study, the MN cells were analyzed using flow cytometry and confocal imaging followed by quantitative image analysis. MN cells had mitotic and chromosome attachment aberrations, multiple centrosomes and micronuclei. These aberrations are known to occur in tumor cells, a finding that links Ccdc124 to cancer. MN cells also upregulated the p53 protein, which induced senescence. Furthermore, MN cells had increased numbers of 53BP1 foci which indicates that the mutation of Ccdc124 induces the DNA damage response and activates the p53 pathway. This study documents a relationship between Ccdc124 mutation-associated cytokinesis failure and p53-dependent senescence.

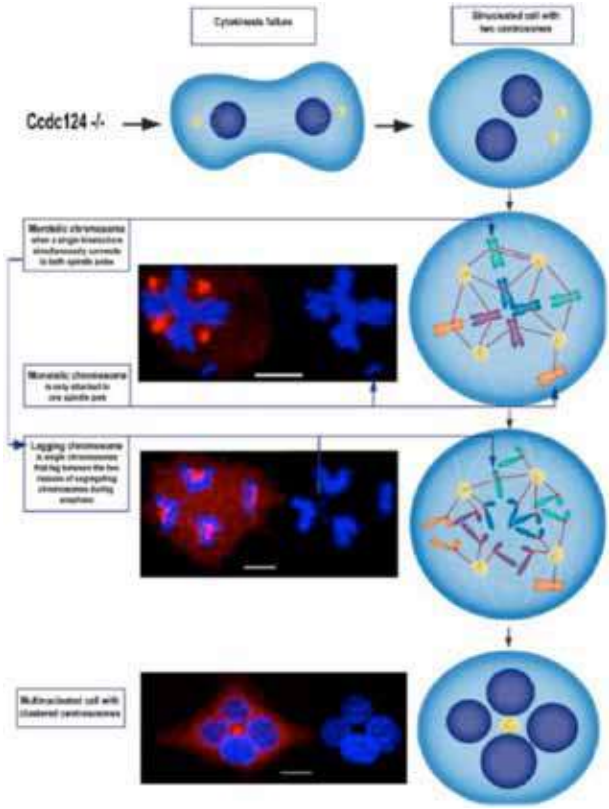
Keywords: Ccdc124; HEK293T; midbody; centrosome; confocal microscope.



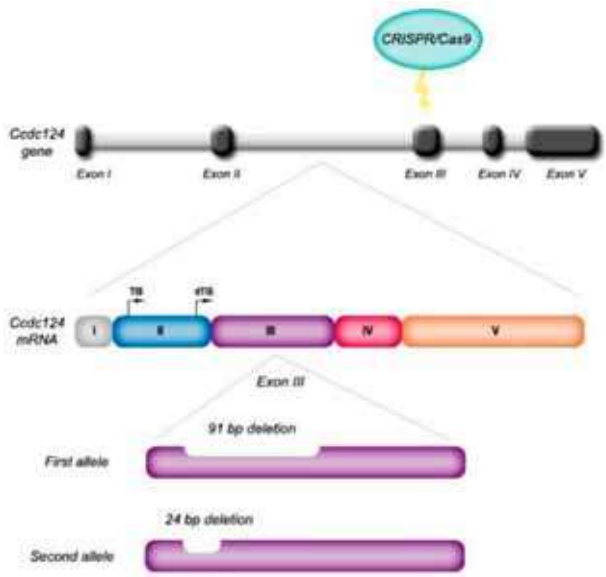
Ccdc124 protein subcellular localization.



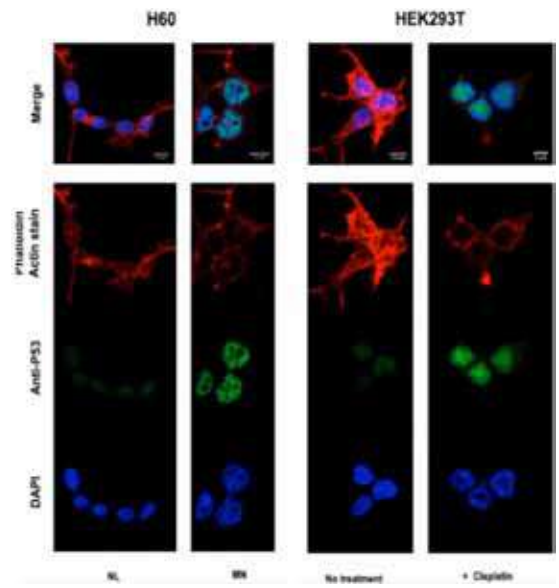
Micronuclei formation in the multinucleated cells.



Chromosomal attachment aberrations.



Mutation of Ccdc124 gene.



p53 upregulation.

[P-0050]

[Immunodeficiencies: Emerging Molecular & Cellular Therapies. Remembering Prof. Isıl BARLAN]

Three novel mutations in CYBA among 22 Iranians with chronic granulomatous disease

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Background: Chronic granulomatous disease (CGD) is a rare primary immunodeficiency (approximately 1 case in 200.000-250.000 newborns) caused by defect in one of the components of nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase enzyme (membrane-bound glycoproteins gp91, and cytosolic subunits p47-phox, p67-phox, p40-phox and p22-phox). This leads to defective intracellular killing by phagocyte cells. One of the components of NADPH-oxidase is p22-phox which is encoded by CYBA gene. In this study were identified new genetic changes of CYBA in 22 Iranian patients with autosomal recessive-CGD (AR-CGD).

Methods: Twenty two CGD patients based on defect in NADPH oxidase activity (by NBT slide test and DHR-123 assay) and their demographic data and clinical histories were entered this study. They were referred to Immunology, Asthma and Allergy Research Institute (IAARI) for diagnosis and treatment. All patients had p22-phox deficiency based on western blotting. Genomic DNA was extracted from peripheral blood mononuclear cells (PBMCs) and PCR followed by direct sequencing to find p22-phox mutations.

Results: Mutation analysis of CYBA revealed 12 different mutations, including three novel mutations: one deletion in exon 1, and two transitions in exon 3 [c.136G>A (p.Gly46Ser)], and exon 6 [c.388C>T (p.Gln130X)].

Conclusions: Three new mutations of CYBA gene in 4 of 22 Iranian patients with AR-CGD were found. These 3 novel mutations can partly complete the data base of Human Gene Mutation Database (HGMD). It can also be helpful for further prenatal diagnosis of next children in the affected families. Also it will be useful for timely decision making in bone marrow transplantation as treatment for CGD patients.

Keywords: Chronic granulomatous disease; p22-phox; CYBA; novel mutation; molecular diagnosis.

[P-0055]

[Regulatory Cells of Immune System]

Modulatory effect of rat bone marrow mesenchymal stem cells on immunological parameters of common bile duct ligated rats

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Introduction: Mammalian liver has highly regenerative capacity following resection or injury by restoring its original mass. However, in the end-stage liver disorders, regeneration usually fails and orthotropic liver transplantation (OLT) seems to be the only curative approach. Cellular therapy is a promising approach that may preclude the need for OLT. Adult or embryonic hepatocytes and mesenchymal stem cells (MSCs) can be used for this purpose. Among them, MSCs are extrahepatic stem cells derived from bone marrow, adipose tissue, pancreatic epithelial progenitor cells, neural and umbilical cord blood-derived somatic stem cells have been shown to possess the potential to trans-differentiate into hepatic cells.

Aim: To show the comparative, regenerative and curative effect of bone marrow MSCs on rats having hepatic fibrosis produced by common bile duct ligation (CBDL) model.

Methods: Rats were divided into three groups; 1- CBDL rats that were given MSCs (CBDL+MSC), 2- CBDL rats that were given phosphate-buffered saline (PBS) (CBDL+PBS), 3- Healthy rats that were sham operated and given MSCs (Healthy+MSCs). Effect of MSC treatment were measured at three levels: morphological, phenotypical and functional. We analyzed the *in vivo* functional. Morphologically, MSCs were labeled with GFP to check the localization of stem cells and to get an idea for the regenerative capacity in the injured liver. Phenotypically, immunological studies were carried out to highlight the immune-regulatory effect of stem cells as to prevent apoptosis of hepatocytes, effects on the levels of regulatory T cells and pro-inflammatory T helper subsets such as Th1. Cytokine secretions from anti-CD2, anti- CD3 and anti-CD28 stimulated splenocytes (lymphocyte subtypes in spleen) were evaluated.

Results: Histologically, liver fibrosis developed in CBDL rats while the entire group of healthy rats did not show any alteration in liver architecture. MSCs suppressed the rat splenocyte proliferation significantly more in CBDL+MSC compared with CBDL+PBS group ($p<0,05$). NK cells in peripheral blood increased significantly more in CBDL+MSC compared with CBDL+PBS ($p<0.05$). Peripheral $CD4^+$ $CD25^+$ ratio increased in CBDL+PBS compared with CBDL+MSC. MSCs suppressed the proinflammatory cytokine levels in CBDL+MSC.

Discussion: Our findings suggest bone marrow derived MSCs may be effective in alleviating the hepatic injury by suppressing the spelonocyte proliferation, increasing the circulating peripheral NK cell population and CD4+CD25+ cells and by suppressing the proinflammatory cytokines in rats. Thus, MSC injection treatment may appear promising in liver injury and future clinical therapies are warranted.

Keywords: Hepatic regenerative medicine; cellular therapy; bone marrow-derived stem cells; common bile duct ligated cirrhotic model; rat.

[P-0056]

[Emerging Issues in Adaptive Immune System]

Pyruvate supplementation and plasma treated culture surfaces increase the number of immature thymic epithelial cells and their stemness ability in human thymic cell culture

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Introduction: Aged population have to struggle with aging associated diseases as an indirect result of immunosenescence, therefore many scientists have concentrated on the regenerative immunology to reboost the immune system in older people. In association with immunosenescence, thymus which is the main site for maturation and selection of T cells, decrease in size and functionality. T cell maturation in thymus, mainly occurs through interaction between immature T cells and thymic epithelial cells (TECs). Determination of ideal conditions for thymic epithelial cells culture is important approach for future regenerative therapies. In our study, we analyzed the effects of method of thymic cell suspension preparation, supplements in culture medium and culture surfaces on TECs in culture.

Materials and Methods: Infant thymuses were homogenized with mechanical homogenization or mechanical homogenization plus dispase treatment. Homogenates were passed through 40µm pore size cell strainer. Cells and tissue parts on the cell strainer were recovered with back wash and they were used for culture. Cells and small tissue fragments (1 mm) from thymus were seeded together in plasma treated polystrene or poly-L lysine coated culture flasks. Cell were cultured with 10% or %1 FBS and 1% or no pyruvate containing medium. Passaged cells were characterized with flow cytometry. CD45neg CD326$^+$ cells were gated as total TECs. CD45neg CD326$^+$ CD205$^+$ cells were identified as cTECs while CD45neg CD326$^+$ CD205neg cells were accepted as mTECs. HLA-DR was used as maturity marker, negative cells were determined as immature while positive cells were determined as mature. For late passage numbers (>5), cells were seeded with 20 ng/mL BFGF, 20 ng/mL EGF, 1xN2 and B27 supplemented culture medium on agarose coated plate for thymosphere formation analysis. Sphere formation assay were used for stemness analysis *in vitro*.

Results: Proliferation of TECs on culture flasks started from the seeded tissue parts. Mechanical treatment maintain TEC morphology and give the highest ratio of immature TECs for late passages (p=5). Pyruvate help the maintenance of TEC characteristics and viability. Cultured cells (p=5) on plasma treated polystrene surface have the 1.6 fold higher immature TECs ratio than cultured cells on poly-L lysine coated surface. Dispase treated cells did not formed spheres while mechanically treated cells formed large spheres.

Discussion: Combination of mechanical treatment, plasma treated surfaces and pyruvate supplementation give the highest immature TEC numbers and stemness. These findings will help for optimized thymic cell culture conditions for the purpose of immune rejuvenation.

Keywords: Flow cytometry; sphere; TEC culture; thymic epithelial cell culture; thymosphere; thymus.

[P-0057]

[Regulatory Cells of Immune System]

The investigation of immunological effects of dental follicule mesenchymal stem cells on house dust mites sensitive asthma patients' lymphocytes *in vitro*

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Aim: In this study, we aimed to investigate the immunomodulatory effects of human dental follicle mesenchymal stem cells (hDF-MSCs) pre-stimulated with/without IFN-γ; in Derp p 1 (+) asthma patients lymphocytes *in vitro*.

Methods: Dental follicles were obtained from University of Marmara Faculty of Dentistry Oral and Maxillofacial Surgery Department. Dental follicle derived MSCs were isolated, characterized and differentiated into osteogenic, adipogenic, chondrogenic lineages. Peripheral blood mononuclear cells (PBMC) were isolated from healty (hPBMC) and Derp 1 + asthma patients (aPBMC). hPBMCs and aPBMC were cultured with and without hDF-MSCs. All cultures were stimulated with Derp 1, dexametasone and anti -CD2, anti -CD3 anti- CD28 (CDmix), pre and post stimulated IFN-γ. Lymphocyte proliferation, Annexin V/PI apoptosis and CD4$^+$ CD25$^+$ FoxP3$^+$ regulatory T cell ratio were analyzed after 72 hours of culture period.

Results: Derp 1 and CDmix stimulated hDF-MSCs suppressed lymphocyte proliferation, apoptosis and enhanced CD4$^+$ CD25$^+$ FoxP3$^+$ T regulatory lymphocyte (Treg) compared to PBMC cultures without MSCs. Pre IFN-γ stimulated hDF-MSCs (24 hours before co-culture) suppressed Derp 1 and CDmix stimulated lymphocyte proliferation, apoptosis and enhanced CD4$^+$ CD25$^+$ FoxP3$^+$ Treg levels compared to hDF-MSCs and on time IFN-γ stimulated hDF-MSCs.hDF-MSCs suppressed CD4$^+$ T cell proliferation significantly ($p<0.05$). Previously stimulated hDF-MSCs with IFN-γ suppressed CD4$^+$ T lymphocyte proliferation more than unstimulated MSCs (p=0.01). hDF-MSCs increased T regulatory cells ratio significantly ($p<0.05$). IFN-γ stimulation of hDF-MSCs enhanced T regulatory cell ratio compared to unstimulated MSCs (p=0.01). Both IFN-γ stimulated and unstimulated hDF-MSCs suppressed lymphocyte apoptosis significantly ($p<0.05$).

Conclusions: hDF-MSCs + suppressed lymphocyte proliferation and this suppression was increased with pre IFN-γ stimulation of hDF-MSCs. IFN-γ pre-stimulated hDF-MSCs suppressed lymphocyte proliferation, apoptosis and increased the number of CD4$^+$ CD25$^+$ FoxP3$^+$ Treg cells of Derp 1 + asthma patients' lymphocytes. IFN-γ stimulation of hDF-MSCs has more suppressive effect on CD4$^+$ T cell response by enhanced CD4$^+$ CD25$^+$ FoxP3$^+$ Treg cells. According to the results, it has been shown that hDF-MSCs suppressed the proliferation of lymphocytes isolated from derp1 (+) asthmatic patients. In accordance with the results it would be appropriate to the IFN-γ stimulated hDF-MSCs can be used in the treatment of inflammatory and allergic diseases to modulate immune responses.

Keywords: Allergic asthma; house dust mites; dental follicle; Derp1; immunomodulation.

[P-0061]

[Inflammation and Diseases]

Increased monocyte toll-like receptor- 2 and 4 expression in atrial fibrillation

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Background: Inflammation contributes to the atrial fibrillation (AF) pathogenesis. Among immune cells, monocytes have been suggested to be involved. Few studies have investigated the monocyte Toll- like receptor (TLR)- mediated inflammation in AF patients.

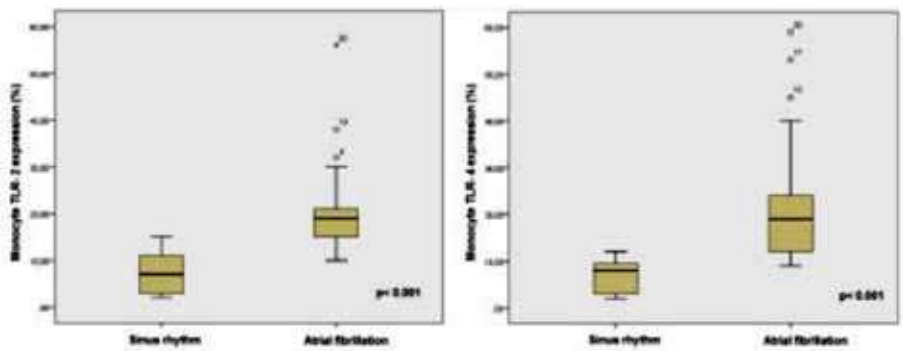
Purpose: In this study, we aim to assess monocyte TLR expression in patients with atrial fibrillation.

Methods: 30 patients with non- valvular AF and 31 healthy patients in sinus rhythm referred for routine check- up were included. Monocyte TLR-2 and 4 expression were evaluated by flow cytometric analysis in peripheral venous blood samples.

Results: 61 subjects (55.7% male, 50.2±11.1 years) were included in the study. Monocyte TLR-2 expression was higher in patients with AF when compared to those in sinus rhythm [18 (10-56) vs. 7 (2-15)%, p<0.001]. Patients with AF also had higher expression of TLR-4 when compared to those with sinus rhythm [19 (9-59) vs. 8 (2-12)%, p<0.001] (Figure).

Conclusion: Our study shows that TLR-2 and 4 expression on monocytes is increased in atrial fibrillation. Elucidating underlying exact pathophysiological mechanisms merit further studies.

Keywords: Monocyte; toll like receptor 2; toll like reseptor 4; atrial fibrillation.



Monocyte TLR- 2 and TLR-4 expression in patients with AF.

[P-0066]

[Cellular and Molecular Targets in Rheumatoid Arthritis and Autoimmune/Autoinflammatory Diseases]

Assessment of platelet count and mean platelet volume rates in patient with active *Helicobacter pylori* infection

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Multiple publications have reported a role for *Helicobacter pylori* infection in causing a variety of extra-intestinal manifestations such as idiopathic thrombocytopenic purpura, systemic lupus erythematosus, rheumatoid arthritis. It has been claimed that *H. pylori* eradication therapy could be used to treat infected patients with immune thrombocytopenic purpura. In some reports findings among H. pylori, platelet volume and platelet count are controversial. The aim of the study was to analyze platelets count and mean platelet volume (MPV) rates in patient with active *H. pylori* infection.

Table 1. Comparison of age, gender, PLT and MPV values between control and patient groups

Study groups	n	Age Mean (±SD)	Gender Female (%)	Platelet count Mean (±SD)	MPV Mean (±SD)
Control	142	41.68 (12.44)	84 (59.2)	255126 (54271)	8.16 (0.70)
Patients	126	38.81 (15.59)	79 (62.7)	278317 (57866)	9.82 (0.93)
P value*		0.095	0.553	0,001	0.000

* Independent samples t-test.

In a retrospective manner, 126 adult patients that stool *H. pylori* antigen test were positive and have dyspeptic complaints who have administered gastroenterology were included the study as patient group. Laboratory results extracted from medical records. Inclusion criteria were having a complete blood count and a positive H. pylori stool antigen test in same day. A total of 142 adult healthy individuals' complete blood counts who administered to hospital as a blood donor were included to study as control group. *H. pylori* stool antigens were detected by a immuno-chromatic commercial kit (HpSA, TOYO) according to manufacturer's directions and CBC test was performed by Mindray cell counter (BC 6800).

The data of study groups are summarized in Table 1. There was no significant difference of age and gender between control and patients groups. The average value of platelets and MPV were both found to be significantly higher compared to patients in the control group.

Although, we need well designed further studies, in a similar manner to the some literature we think that the MPV and platelet count may be affected by active *H. pylori* infection.

Keywords: Helicobacter pylori; MPV; platelet count;, immune thrombocytopenic purpura.

[P-0067]

[Emerging Issues in Adaptive Immune System]

Ape1/Ref1 facilitates gene conversion in DT40 cells

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Antibody affinity maturation in mammalians is enabled by mainly two different mechanisms, gene conversion and somatic hypermutation. They initiate by a common molecular intermediate on DNA including abasic sites. It has been envisaged that strand breaks were also required, however it was not confirmed yet. In the present study, we addressed this question in DT40 cells by suppressing Ape1/Ref1, an endonuclease which processes abasic sites into strand breaks. We used a mutant form of this protein called ED and it was reported that ED binds to DNA much stronger than its wild type form and prevents strand break formation when it is over-expressed. We started our experiments by transfecting ED into DT40 cells by electroporation. Initial observations with flow cytometry indicated that gene conversion was indeed blocked up to 90%, suggesting Ape1/Ref1 is required for gene conversion in chicken DT40 cells. In conclusion, our results strongly support the activity of Base Excision DNA Repair in defense against invaders during B cell mediated adaptive immunity.

Keywords: Antibody maturation; gene conversion; base excision repair; DT40 B cells; Ape1/Ref1.

[P-0068]

[Inflammation and Diseases]

Immune dysregulation in peripheral blood cells of patients with idiopathic pulmonary arterial hypertension

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Idiopathic pulmonary artery hypertension (iPAH) is a complex, multifactorial and fatal disease characterized by profound pulmonary artery remodeling. The role of the immune system on its pathogenesis remains poorly understood, however due to the accepted impairment of endothelium, immune system contribution may be of interest. This study aims to investigate the immune parameters, which might be correlated with the disease pathology.

Heparinized peripheral blood samples were obtained from iPAH patients (n=5) and healthy subjects (n=6) and the expression of CD3, CD4, CD8, CD19, CD16, CD56 and HLA-DR were measured by flow cytometry. The peripheral blood mononuclear cells were isolated from blood samples and cultured for 72h and 120h with IL-2, IL-12, TGF-β and IL-2+IL-12 cytokine stimulations. The ratios of CD16+CD56dim and CD16-CD56bright NK cells and their CD49d expression were detected at 72h and CD16+CD56dim and CD16-CD56bright NK cell proliferation were determined at the 120h of cell culture.

Significantly decreased ratios of CD19+ and CD3+HLA-DR+ cells as well as increased NK cells were observed in iPAH patients. Decreased proliferative capacity of CD16-CD56bright cells in response to IL-12 and TGF-β was observed in patients, compared to healthy subjects.

Our findings support the possible role of immune system in the pathophysiology of iPAH. A better understanding of its contribution would help to improve treatment options, which may prolong the life expectancy of patients with iPAH.

Keywords: NK; pulmonary arterial hypertension; pathogenesis.

[P-0069]

[Cellular and Molecular Targets in Rheumatoid Arthritis and Autoimmune/Autoinflammatory Diseases]

Polymorphism of heat shock protein 70 genes in pemphigus foliaceus: a possible role of {HSA1A}, {HSPA1B} and {HSPA1L} variants

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The human Heat Shock Proteins (HSP70) family plays a key role in up-regulating stress responses. Some studies reported possible associations of single nucleotide polymorphisms in the HSP70 genes with some autoimmune diseases. However, whether HSP70 polymorphisms represent a risk factor for pemphigus foliaceus (PF) is still unkown.

We analyzed by PCR-RFLP polymorphisms of HSP70 genes HSA1A, HSPA1B and HSPA1L in 80 Tunisian patients with PF, 160 matched healthy controls and 147 related healthy subjects.

There were significant differences between PF patients and controls in the allelic (pc=5.91 10-12, pc=1.14 10-5 and pc=0.0089; respectively) and homozygous genotypic frequencies of HSPA1L>T, HSPA1A>C andHSPA1B>G (p=2.61710-12, p=1.017 10-5 and p=0.0058; respectively). Decreased serum and mRNA levels of HSP70 may have an effect on epidermis under heat or UV radiation exposure and the HSPA1L>T/T, HSPA1A>C/C and HSPA1B>G/G genotypes found in our present study as associated with PF, may be related to the disease through similar mechanisms. Such alteration may impair their ability to assemble and transport some newly synthesized proteins within cells, as well as to remove denatured proteins that may be formed as a result of damage like in PF disease which can lead to the intracellular accumulation of denaturalized proteins or peptides transporting defects affecting self tolerance and may cause autoimmunity by molecular mimicry. According to these findings, the lower production of HSP70 proteins could explain in part the frequency of this endemic form of PF in these south regions of Tunisia recognized by temperature and UV radiation intensity particularly high. An interaction between environmental and genetic predisposing factors is thought to be involved in the etiology of PF. Indeed, environmental factors may induce processes in the skin which promote the exposure of self-antigens and the development of subsequent and progressive humoral autoimmunity.

Haplotype analysis showed significant differences between PF patients and controls: the CCA, CGA, CCG and CGG haplotypes were significantly over-represented in controls whereas the TCG haplotype was significantly over-represented in patients indicating that they may contribute to the susceptibility to skin damage induced by exposure to environment stress such as high temperature.

However, the significant LD found between the HSP70 and the HLA class II susceptibility alleles together with the multivariant regression analysis data between the two loci could argue against a direct role of the HSP70 polymorphism in the occurrence of PF.

Keywords: Pemphigus foliaceus; heat shock protein 70; gene; polymorphism; HLA-DR3; Tunisia.

Figure 1. **(a)** The human susceptibility locus on chromosome 6p21.33 covers a cluster of three Hsp70 genes: Hsp70-hom (HSPA1L), Hsp70.1(HSPA1A) and Hsp70.2 (HSPA1B). **(b)** Pairwaise LD analysis of PF susceptibility loci corresponding to the Lewontin.

[P-0070]

[Regulatory Cells of Immune System]

The cytokine secretion and the regulatory role of CD4⁺T Cells in ciprofloxacin-related drug reactions

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Objective: Ciprofloxacin (CPFX) is a widely used quinolone antibiotic which induces cutaneous adverse drug reactions in about 1% to 2% of treated patients. Definite diagnosis of delayed type hypersensitivity reactions (DTHR) may be challenging; however the lymphocyte transformation test (LTT) can be beneficial for *in vitro* diagnosis of drug hypersensitivity reactions. The aim of this study was to evaluate the activation markers, intracellular cytokine secretion and LTT of drug stimulated CD4⁺ T cells, and additionally IL-4, IL-10, IL-2 and IFN-γ λεῳελσ in plasma and CPFX-stimulated culture supernatants.

Methods: Patients who experienced DTHR (3 maculopapular eruption, 1 fixed drug eruption, 4 delayed onset urticaria) to ciprofloxacin (n=8, mean age: 45±12 years) and healthy subjects (n=10, mean age: 47±13 years) were included in the study. Intradermal tests and patch tests with CPFX were performed. LTT was analyzed in order to determine CPFX-specific CD4⁺ T cells by flow cytometry. CD25, CD28, CD69 and HLA-DR expression of CD4⁺ T cells and intracellular IL-4, IL-10, IL-2 and IFN-γ levels were studied in CPFX-specific CD4⁺ T cells by flow cytometry. IL-4, IL-10, IL-2 and IFN-γ levels in plasma and drug stimulated culture samples were analyzed by ELISA.

Results: Intradermal tests with CPFX were positive in only one patient and patch test results were negative in patients. CD25 and HLA-DR expressing CD4⁺ T cells were increased in patients. Compared to unstimulated conditions and healthy subjects, CD4⁺ T cell proliferation significantly increased in patients stimulated with CPFX. Analysis of cytokine levels in CPFX stimulated PBMC cultures revealed a decreased IL-10 and an increased IL-4 in study group, IFN-γ and IL-2 levels were found statistically similar. The percentages of CD4⁺ IL-2⁺ and CD4⁺ IL-4⁺ T cells were significantly increased and IL-10⁺ CD4⁺ T cells were significantly decreased in patients with DTHR in CPFX stimulated cultures. Moreover increased plasma levels of IL-2 and decreased IL-10 and IFN-γ levels in patients were detected.

Conclusion: Our findings suggest that the increase of IL-2 and IL-4-secreting CD4⁺ T cells together with decreased IL-10 secreting CD4⁺ T cells are associated with quinolone-related delayed type hypersensitivity reactions. Reduced IL-10 levels in plasma, CPFX-stimulated cultures and also IL-10⁺ CD4⁺ T cells demonstrate the loss of regulation, which may be due to the DTHR. We suggest that intracellular cytokine measurement with LTT can support the diagnosis of quinolone hypersensitivity when the *in vitro* tests remain negative.

Keywords: Drug allergy; CD4⁺ T cells; ciprofloxacin; LTT; intracellular cytokines.

[P-0072]

[Inflammation and Diseases]

Agammaglobulinemia developing after rituximab treatment

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Rituximab is an anti-CD20 B cell depleting monoclonal antibody and a therapeutic agent used for a wide range of autoimmune disorders, lymphoma and for patients with refractory disease with other immunosuppressive treatments. Rituximab causes significant depletion of CD20-expressing B cells in lymphoid tissue, in circulation and modificates B cell maturation in the bone marrow. Consequently, hypogammaglobulinemia is noted after rituximab therapy. Herein, we report two patients developing after rituximab treatment for their primary disorders.

Case 1: An 11-year-old female patient, having the diagnosis of end stage renal failure secondary to Antineutrophil cytoplasmic antibody (ANCA) associated glomerulonephritis. She had received steroid and cyclophosphamide before but rituximab therapy was obligated to start because of refractory disease. She developed agammaglobulinemia in the first year of treatment.

Case 2: A 20-year-old female patient had the history of renal transplantation for chronic renal failure. Unfortunately she had developed Non-Hodgkin's lymphoma (NHL) in the second year of transplantation and had received 6 doses of rituximab for NHL. Resistant candida infections and agammaglobulinemia occured one year after starting rituximab.

For both of the patients serum immunoglobulin levels were normal before rituximab treatment.

Patients are at risk of developing hypogammaglobulinemia after rituximab treatment, however the incidence and clinical significance of rituximab induced hypogammaglobulinemia is ignored. We suggest the importance of following serum immunoglobulin levels for patients receiving rituximab.

Keywords: Rituximab; agammaglobulinemia; renal failure.

[P-0073]

[Inflammation and Diseases]

The serum levels of resistin and its relationship with other proinflammatory cytokines in patients with Alzheimer's disease

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The aim of this study was to analyze the levels of resistin, tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, IL-6, IL-18, and C-reactive protein (CRP) in patients with Alzheimer's disease (AD) and also to assess the possible relationship between the resistin level and the TNF-α, IL-1β, IL-6, IL-18, and CRP levels in AD patients. Fifty patients with AD and 30 healthy controls with normal cognitive function were enrolled in this study. The serum resistin, TNF-α, IL-1β, IL-6, IL-18, and CRP were evaluated. Mini-Mental State Examination (MMSE) was performed for evaluation of the general cognitive performance. The mean serum resistin, IL-1β, IL-18, and TNF-α levels were significantly higher in patients with AD compared with the controls (p=0.026, p=0.002, p=0.003, p=0.038, and respectively). The IL-6 and CRP levels were not different between the groups (p=0.874 and p=0.941). The levels of resistin were positively correlated with the levels of CRP and IL-18 (r=0.526, p<0.001 and r=0.402, p=0.004). There was no correlation between the scores of MMSE and inflammatory markers (p>0.05 for all). Serum resistin were significantly increased and correlated with the inflammatory markers in AD patients, suggesting that resistin might play a role in the inflammatory process of AD.

Keywords: Resistin; inflammatory cytokines; Alzheimer's disease; inflammation.

Table 1. The demographic, clinical and laboratory parameters in patients with AD and controls

	AD (n=50)	Controls (n=30)	p
Age (years)	75.8±8.01	73.1±5.73	0.101
Sex (F/M)	31 (62%) /19 (38%)	18 (60%) /12 (40%)	0.859
MMSE	17.7±5.62	27.4±1.19	<0.001
CRP (mg/L)	6.97±6.28	6.62±5.68	0.941
IL-1β (pg/mL)	11.8±51.8	1.69±4.95	0.002
IL-6 (pg/mL)	19.4±88.5	14.0±50.7	0.874
IL-18 (pg/mL)	117.97±133.59	61.5±40.2	0.003
TNF-α (pg/mL)	148.94±202.21	64.0±84.8	0.038
Resistin (ng/mL)	2.42±0.74	2.11±0.56	0.026

AD, Alzheimer's disease; MMSE, Mini-Mental State Examination; CRP, C-reactive protein; TNF-α, tumor necrosis factor alpha; IL, interleukin

Table 2. The correlation between MMSE and laboratory parameters and between resistin and other laboratory parameters

	MMSE	MMSE	Resistin (ng/mL)	Resistin (ng/mL)
	r	p	r	p
CRP (mg/L)	-0.125	0.432	0.526	<0.001
IL-1β (pg/ml)	-0.117	0.419	0.279	0.050
IL-6 (pg/ml)	-0.114	0.431	0.118	0.414
IL-18 (pg/ml)	-0.075	0.603	0.402	0.004
TNF-α (pg/ml)	-0.206	0.151	0.128	0.375
Resistin (ng/mL)	-0.198	0.169	-	-

AD, Alzheimer's disease; MMSE, Mini-Mental State Examination; CRP, C-reactive protein; TNF-α, tumor necrosis factor alpha; IL, interleukin

[P-0074]

[Transplantation Immunology]

Effects of sample preservation conditions on DNA isolation

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Aim: Even though quality of DNA analysis depends on how blood samples are stored, methodological differences also affect quality of analysis. In this study, we isolated DNA from blood samples stored in different conditions (commercial RNA preservation solution, mononuclear cells isolated with density gradient and nucleated cells obtained through whole blood lysis).

Method: Blood samples were collected from 11 patients who underwent lung transplantation, taken at 3 different time points: before transplant, 24 hours and 7 days after the procedure. 33 blood samples; stored in commercial RNA preservation solution or isolated mononuclear cells by either density gradient separation or lysing erythrocytes were stored at -80 °C for a year. DNA isolation was performed and samples were evaluated for DNA quality with 260/280 absorbance ratios measured in spectrophotometry.

Results: First run of DNA analysis was performed on 18 whole blood samples stored in commercial RNA preservation solution and isolated with 3 different methods: DNA isolation robot, commercial kits, and manual DNA isolation methods (extraction from TRIzol and CTAB-DTAB). DNA analysis was performed on 15 samples' which cells were separated either by density gradient or lysing erythrocytes isolated by 2 different manual methods: extraction from TRIzol and CTAB-DTAB. Among all storage conditions and DNA isolation methods, DNA extraction from TRIzol with isolated cells (either with density gradient or erythrocytes lysis) was found most successful.

Conclusion: For molecular analysis, it is important to do validation and verification at every step from the beginning of sample collection. In this study, we got best results with extracting DNA from TRIzol with mononuclear cells stored in Voluven + DMSO among all blood samples. We expect that our methodological approach for validation of pre-analytic phase of this study to be inspiring for research or diagnostic purposes.

Keywords: DNA isolation; TRIzol; sample preservation.

[P-0076]

[Regulatory Cells of Immune System]

Aneuploidy induced by the mutation of the Ccdc124 gene

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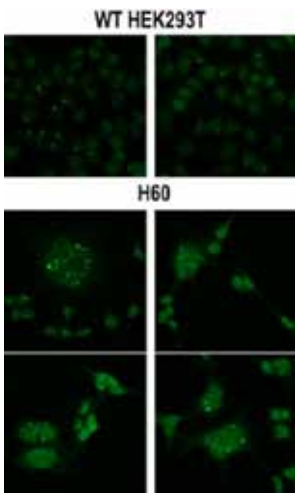
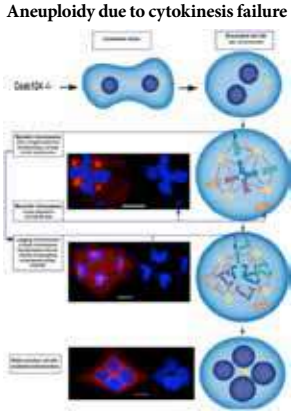
53BP1 foci formation



53BP1 foci number was increased in the Multinucleated cells in comparison to the WT cells, which can be the cause of p53 activation as a result of DDR response, 53BP1 foci formation is a marker of DNA damage which can be induced by CIN that is accompanied by aneuploidy in most cases, due to increase number of chromosome attachment errors.

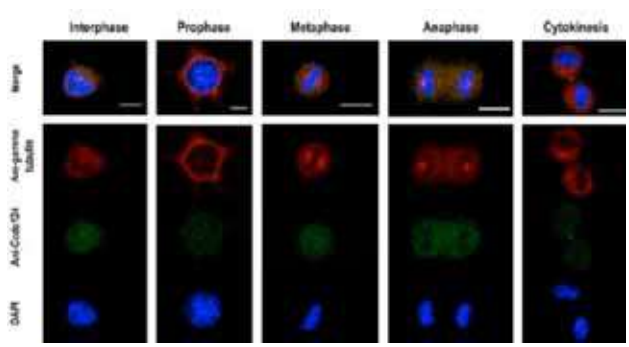
The Coiled-coil domain containing protein 124 (Ccdc124) is a centrosomal protein that relocates to the midbody region at the cytokinesis stage of the cell cycle. Cytokinetic abscission is the cellular process that leads to physical separation of two postmitotic sister cells by severing the intercellular bridge. Mutation of the Ccdc124 gene by CRISPR/Cas9 genome editing in HEK293T cells leads to the failure of cytokinesis and formation of aneuploid (multinucleated-MN) aberrant cells. In this study, the MN cells were analyzed using flow cytometry and confocal imaging followed by quantitative image analysis. MN cells had mitotic and chromosome attachment aberrations, multiple centrosomes and micronuclei. These aberrations are known to occur in tumor cells, a finding that links Ccdc124 to cancer. MN cells also upregulated the p53 protein, which induced senescence. Furthermore, MN cells had increased numbers of 53BP1 foci which indicates that the mutation of Ccdc124 induces the DNA damage response and activates

Aneuploidy due to cytokinesis failure



Aneupoidy is associated with chromosomal instability (CIN), such as chromosome attachment errors.

CCEC124 protein

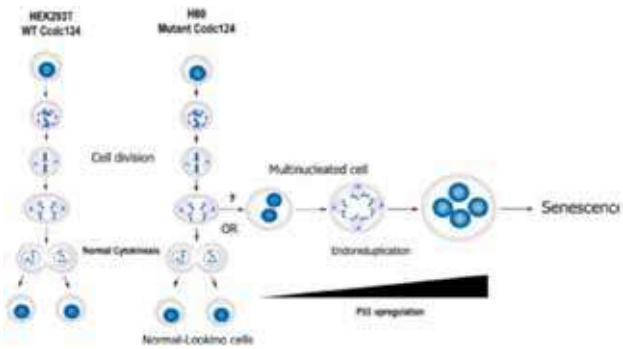


Ccdc124 protein subcellular localization at centrosomes in interphase and prophase then translocation to midbody region at cytokinesis stage in the middle of the two separating daughter cells.

the p53 pathway. This study documents a relationship between Ccdc124 mutation-associated cytokinesis failure and p53-dependent senescence. We also discuss the significance of the CCDC124 protein in T lymphocytes, as the defects we observe are related to the microtubule organizing center (MTOC) that plays a critical role in T lymphocyte polarization.

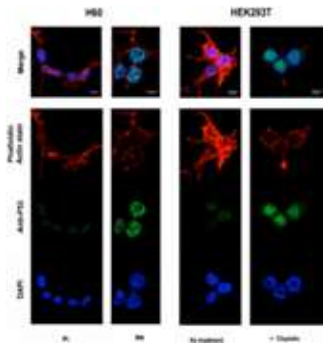
Keywords: Ccdc124; centrosome; midbody; aneuploidy; T lymphocytes.

Model



WT HEK 293T cells undergo normal cytokinesis, whereas the Ccdc124 mutated cells H60 about 80% undergo normal mitosis as well, but about 20% undergo cytokinesis failure which cause aneuoploidy accompanied with P53 upregulation and senescence as a final outcome.

P53 upregulation



P53 upregulation in the aneuploid MN cells, in comparison to the wild type cells.

[P-0077]

[Tumor Immunotherapy]

CRISPR/Cas9 mediated mutation of the p53 protein C-terminus in colon cancer cell lines

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Half of all cancer cases have a mutated p53 gene. The p53 protein's C-terminal domain plays an important role in its post-translational modification. We studied the function of this domain in endogenous p53 in HCT116 human colon cancer cell lines. We generated several C-terminal mutant (CTM) cell lines through CRISPR-Cas9 mediated genome editing. The focus of this study is three of these p53 CTM cell lines that encode 1) a truncated p53 protein (p53 Δ21/Δ21); 2) a single amino acid deleted p53 protein (p53 ΔR379/ ΔR379); and 3) a p53 protein missing the C-terminus encoded by the last p53 exon (p53 Δ26/Δ26). We show that cells which express the p53 ΔR379 protein (with deleted Arg379), grow slower and cannot accumulate p53 as much as wild type counterparts, under stress. This p53 mutant cannot bind to DNA targets as efficiently as WT p53. The p53 Δ21 mutant lacks 21 amino acids at the C-terminus. This mutant p53 can accumulate after DNA damage and can bind to DNA targets efficiently; nevertheless it cannot interact with a recently identified p53 inhibitory protein, PATZ1. The p53 Δ26 expressing mutant cell line lacks the last exon of p53 on both alleles, behaves in a very different way. The phenotype of this mutant is similar to HCT116 -/- cells that do not express p53. We assessed the expression of p53 target genes by quantitative RT PCR and found defects in the expression of these genes. However, these mutant cells express truncated p53 and they grow much slower and go to senescence twice as fast as HCT116 -/- counterparts. This p53 mutant cannot translocate into the nucleus after DNA damage induced by Doxorubicin treatment. These results indicate that the p53 C-terminal domain is very critical for the protein's function.

Keywords: p53 C-terminal domain; CRISPR/Cas9; colon cancer.

[P-0079]

[Tumor Immunology & Molecular Markers]

Decreased cytokine content in conditioned medium of hMSCs reduces the tumorigenicity in cancer cells

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Tumor surrounding tissue carries a high number of mesenchymal stem cells (MSCs) which exert anti-inflammatory and immunomodulatory effects via secretion of a wide range of cytokines and chemokines. A high number of studies suggest that MSCs could facilitate tumor progression and drug-resistance by direct cell-cell contact, immune system suppression and secretion of cytokines. The mechanism by which MSCs promote tumor progression is largely unknown but positive or negative effects of MSCs on tumor sites heavily depend on their secretome. Therefore controlling MSC secretome is significant for utilisation of these cells in the prospective targeted therapies. Since common therapeutic agents are known to enhance cancer cell resistance in cell-based therapies, there is an urge to investigate novel agents in cancer treatment. Here, we aimed at investigating the effect of active compounds, myrtucommulone-A (MC-A) and thymoquinone (TQ), on hMSC cytokine expression. Our data revealed that, MC-A, but not TQ treatment has significantly altered cytokine expression in hMSCs. Upon MC-A treatment, TNF-α, VEGF, IL-6, IL-8 and FGF-2 expression levels were decreased in hMSCs. hMSC conditioned medium (CM) primed with MC-A decreased the proliferation, migration ability and clonogenicity of bladder and breast cancer cells in comparison to non-primed hMSC medium and hMSC medium primed with TQ. In conclusion, our study demonstrates that cytokine expression of hMSCs could be harnessed by MC-A treatment which illuminates the potential of novel anti-cancer agent in targeted MSC-based therapies.

Keywords: Human mesenchymal stem cells; conditioned medium; myrtucommulone-A; thymoquinone; bladder cancer; breast cancer.

[P-0080]

[Cellular and Molecular Targets in Rheumatoid Arthritis and Autoimmune/Autoinflammatory Diseases]

Immune response characteristics of STING associated vasculopathy with onset in infancy (SAVI) patients

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Response to cytosolic DNA depends on the expression of the adaptor STING, culminating in type I IFN production. Gain-of-function mutations in STING has recently been shown to trigger an interferonopathy characterized by neonatal-onset systemic inflammation with severe cutaneous vasculopathy (STING-associated vasculopathy with onset in infancy, or SAVI) leading to extensive tissue loss and interstitial lung disease. Herein, we present data on immune activation status of 3 new cases of SAVI syndrome. Genetic analysis by direct sequencing revealed N154S mutation in one patient and a novel compound heterozygous mutation of V155E/L170Q in TMEM173 in 2 others. N154S mutation results in constitutive activation of the STING-IRF3. qRT-PCR results demonstrate that unstimulated PBMC of the patient with N154S mutation have significant upregulation of interferon stimulated genes (ISGs) compared to healthy controls, while patients with V155E/L170Q mutations showed moderate level of upregulation of ISGs, suggesting that differences in STING-TBK1-IRF3 axis may exist in various TMEM173 mutations that may affect clinical features of this disease. Similar results were found in plasma IP-10 levels where patients have significantly higher levels of IP-10 compared to healthy controls. To assess cGAS activity, monocytes from HSV DNA stimulated samples were co-cultured with a STING reporter cell-line and gap-junction-mediated intercellular cGAMP transfer was determined. Results showed that DNA transfection stimulated patient cells to synthesize and then transfer cGAMP to the reporter cells, suggesting that cGAS activity was not compromised in SAVI syndrome. Collectively, these results could contribute to our understanding of mechanisms that contribute to the pathogenesis of this rare disease.

Keywords: SAVI; STING; cGAMP; interferonopathy.

[P-0081]

[Immunodeficiencies: Emerging Molecular & Cellular Therapies. Remembering Prof. Isıl BARLAN]

The distribution of NK subsets in CVID and some primary immunodeficient patients

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Primary immunodeficiencies are genetic disorders in which part of the body's immune system is missing or does not function normally. PID patients show predisposition to infection as well as allergy, autoimmunity and cancer. Natural killer (NK) cells are a component of innate immune system and play a major role in the host-rejection of tumors and virally infected cells both. NK cells can be divided into two subsets based on their cell-surface density of CD56 as "CD56bright" and "CD56dim". The CD56dim NK-cell subset is more naturally cytotoxic and expresses higher levels of Ig-like NK receptors and Fcγ receptor III (CD16) and synthesizes perforin. By contrast, the CD56bright subset has the capacity to produce abundant cytokines. The aim of this study is to analyze by Flow Cytometry peripheral blood NK cells number and functions in patients with Common Variable Immunodeficiency (CVID), Hyperimmunglobulinemine M syndrome (hyper IgM), ataxia telangiectasia (AT) and agammaglobulinemia (AG).

Peripheral blood samples obtained from patients (CVID n=7, Hyper IgM n=2, AT n=4 and agammaglobulinemia n=4), and NK cells were evaluated using anti-CD3, -CD4, -CD16, -CD56, -NKp30, -NKp44, -NKp46, -NKG2D, -perforin and anti-granzyme monoclonal antibodies and compared with healthy individuals (n=3) according to whole blood lysing protocol. Cell surface (NKp30, NKp44, NKp46, and NKG2D) and intracellular molecules (perforin and granzyme) expressions were analyzed in lymphocyte, CD3- CD16+ and CD3- CD56+ gates.

CD3 cells were decreased in AT and CVID patients compared with AG patients (p=0.020 and p=0.005 respectively). CD3- CD16+ CD56dim and CD3- CD16+ NK cell subsets were decreased in AG and CVID patients compared with AT patients (p=0.020 and p=0.010 respectively). NKp30 expressions were decreased in CVID patients than healthy individuals (p=0.03) and in AG than AT patients (p=0.030). Low granzyme expression was observed in CVID than AT patients (p=0.005).

It was reported the increase of malignancy in the patients with AT, hyper IgM and AG, but the data of NK cell number and activator/inhibitor receptors are limited in these diseases. These preliminary results demonstrate basal count and distribution of NK cells subset. These patients will be monitored about malignancy progression, and our findings could be shed light on role as defense against the tumor development of these cells in PID clinic.

Keywords: NK cell; primary immunodeficiencies; flow cytometry.

[P-0083]

[Inflammation and Diseases]

Involvement of hemolytic effect of {Androctonus australis hector} venom in kidney pathogenesis: role of inflammatory and oxidative mediators

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Studies performed with several nephrotoxic venoms indicated that interrelated processes, such as direct cytotoxic action, renal inflammation, oxidative damage and hemolysis may contribute to the acute kidney pathogenesis. This study was undertaken to evaluate the intravascular hemolysis and kidney injury induced by Androctonus australis hector (Aah) venom as well as the direct hemolytic activity of this venom. In addition, to elucidate the role of inflammatory and oxidative mediators in these pathological changes, an inhibitor of phohodiesterase with anti-

inflammatory and anti-oxidative activities (pentoxifylline, 50 mg/kg, oral route) was used prior to the envenomation of mice. Intravascular hemolysis biomarkers and biochemical indicators of inflammatory and oxidative stress were measured in blood and renal homogenate. Histopathologic and renal functional analysis were also performed. Obtained results revealed that Aah venom is able to induce a direct hemolytic effect on washed erythrocytes and intravascular hemolysis as indicated by the increase of hemolysis biomarker levels (free hemoglobin, unconjugated bilirubin, lactate dehydrogenase activity and reticulocytes count). Hemolysis was associated with renal tissue damage characterized by hemorrhage, edema and the formation of tubular haemoglobin casts, which are highly nephrotoxic. These alterations were accompanied by neutrophil infiltration into renal tissue and an increase of nitric oxide levels and lipid peroxidation products with antioxidant defence decrease. Results showed also an increase of urea and creatinine levels indicating a dysfunction of the kidney. The pentoxifylline seems to be able to maintain the level of the studied parameters and to reduce the nephotic pathological changes. In conclusion, the present findings could help to better understand the role of free hemoglobin released after hemolysis in scorpion venom-induced acute renal injury and to propose new therapy using as targets inflammatory and oxidative mediators to attenuate the induced renal alterations observed during scorpion envenoming.

Keywords: Scorpion venom; hemolysis; kidney injury; inflammation; stress oxidative; pentoxyfilline.

[P-0087]

[Regulatory Cells of Immune System]

Investigation of CD4+, CD25+ and CD4+, CD25- T cell phenotypes in patients with recalcitrant warts

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Background and Objective: Current data suggest that there are 2 subsets of regulatory T cells (Treg). Treg cells; CD4+, CD25+ naturel Treg (nTreg) cells and induced Treg (iTreg) cells. Naturel Treg cells develop in thymus while induced Treg (iTreg) cells are generated in the periphery after the recognition of antigen by CD+4, CD25- T cells. Treg cells can supress both CD+4 and CD8+ cells which have suppressive effect on warts. We aimed to investigate the ratios of T cell phenotypes CD4+, CD25+ and CD4+, CD25- in patients with recalcitrant warts comparing with healthy controls.

Material Methods: Forty one patients with recalcitrant warts and 42 healthy controls were included in this study. Using flow cytometry, we counted the CD4+, CD25+ and CD4+, CD25- T cells phenotypes in the mononuclear cell populations from peripheral blood samples.

Results: The patients with recalcitrant warts mean Treg CD4+ CD25+ Foxp3+ cells ratio was statistically significantly higher than healthy controls ($p<0.05$). From the aspect of T cell phenotyphe CD4+ CD25- Foxp3+, the mean ratio was statistically significantly lower in the patients with recalcitrant warts than in healthy controls ($p<0.05$). Both CD+4 and CD8+ levels did not statistically significantly differ between patients and controls.

Conclusions: From these data, we conclude that presence of CD4+ CD25+ Foxp3+ T cells phenotype activity has a role in etiopathogenesis of recalcitrant viral warts. Therefore, blockage of Treg cell transformation pathway may be a promising strategy to modulate the clinical course of warts.

Keywords: Recalcitrant warts; regulatory T cells (Treg); CD4+ CD25+ Foxp3+ T cells.

[P-0094]

[Inflammation and Diseases]

CpG loaded fluorescent polymeric nanoparticles: a theranostic drug delivery system appropriate for TLR based applications

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Aim: Designing nanoparticulate delivery systems suitable for simultaneous imaging and induction of immune response is an attractive therapeutic approach. Several systems such as liposomes, micelles, dendrimers, nano-spheres and nano-capsules are potential theranostic carriers in biomedical applications. Here, for the first time we describe a complexation strategy suitable for delivering a TLR ligand along with imaging capabilities of the cells/tissues utilizing a fluorescent polymeric nanoparticle (NPs).

Method: Four different NPs (P1, P2, P3 and P4) were used for biocompatibility and drug delivery experiments. RAW264.7 cells were incubated with NPs for indicated times and analyzed by FACS and confocal microscopy. Cytotoxicity experiments were performed with Dojindo reagent on RAW264.7 cells. In addition, cellular uptake of NPs in the presence of several scavenger receptor ligands was investigated to understand the mechanism of internalization. Next, NPs were incubated with CpG ODN to yield nano-complexes. *In vitro* immune stimulatory action of nano-complexes was analyzed both on PBMCs and mouse spleen cells. Pro-inflammatory cytokine production was assessed either by ELISA or by PCR. *In vivo* immune stimulatory capacity of nano-complexes was assessed by OVA immunization studies in BALB/c mice.

Results: FACS and confocal analyses revealed that NP internalization kinetics was NP dependent. Cytotoxicity experiments demonstrated that none of the four NPs was toxic when up to 100 μg/ml concentrations were used on macrophages. Data revealed that internalization of amphiphilic particles was dependent on scavenger receptors (SRs). When cells were pre-treated with dextran sulfate a ligand for SRs, it blocked >60% of NPs uptake. P1-CpG ODN nano-complex substantially improved CpG ODN mediated IL-6 and IP10 production by PBMCs. Furthermore, following i.p. injection, NP1-CpG ODN nano-complex significantly upregulated expression of TNF-α, MIP1α, MIP1β and several other TLR transcript expressions. Moreover, P1-CpG ODN was also enhanced the strength and nature of anti-OVA immunity in BALB/c mice. IgG2a/IgG1 ratio was found to be more than 1 suggesting presence of Th-1 biased anti-OVA response.

Conclusion: Present study suggested that fluorescent polymeric nanoparticles are well tolerated and can readily form nano-complexes with nucleic acid ligands. NPs are promising candidates not only for cell tracking and imaging applications but at the same time capable of triggering immune activation via its cargo. Our findings suggest that these materials offer great potential for theranostic applications.

Keywords: Fluorescent polymeric Nanoparticles; CpG ODN; immune activation; immunization.

[P-0095]

[Immunodeficiencies: Emerging Molecular & Cellular Therapies. Remembering Prof. Isıl BARLAN]

Identification of transcriptional control elements of the IL7 receptor gene

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The receptor protein (IL7R) that is encoded by the Il7r gene is expressed in immune system cells and it allows these cells to be signaled by the IL7 survival factor. Mutations in the IL7R pathway are associated with primary immunodeficiency diseases. Polymorphisms in the Il7r gene are directly linked to T-cell acute lymphoblastic leukemia (T-ALL) and multiple sclerosis (MS). These findings point to the importance of this signal in health. We have assessed the transcription mechanism of the IL7R gene in T lymphocytes. We aim to identify transcription factors that bind to the Il7r gene control elements, we have generated mutations in the Il7r gene by using CRISPR/Cas9 mediated genome editing. We have targeted Notch and NF-kB binding sites in the enhancer and RORgammaT and Gfi-1 binding sites in intron 2 of the Il7r gene. Targets of the transcription factors binding to the Il7r gene will be determined by chromatin immunoprecipitation (ChIP). With these studies we aim to identify the important transcription factors that control the survival of lymphocytes of the immune system.

This project is supported by TUBITAK project 113S811

Keywords: Interleukin-7 receptor; genome engineering; T lymphocyte; CRISPR technique.

[P-0096]

[Immunity to Infection: New Trends and Developments]

Functional interactions between {Helicobacter}-activated B (Hact-B) cells and CD4+ T cells

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The immune response mechanisms against Helicobacter involve both protective and damaging effects for the host. Helicobacter induces B cells to produce and secrete IL-10 which has a role in the differentiation of naive $CD4^+$ T cells into Tr1 cells; and TGF-β that has an important role in both Tr1 and Th17 differentiation. The experimental results in our laboratory showed that Helicobacter-activated (Hact) IL-10^+ B cells are the source of the IL-10 while Hact-IL-10^- B cells produce TGF-β and IL-6. Hence, in this study, the role of Hact-IL-10^+ B cells and the Hact-IL-10^- B cells on $CD4^+$ T cell differentiation was investigated. $CD19^+$ B and $CD4^+$ T cells were magnetically isolated from spleens of C57BL/6 mice. Splenic B cells treated with *Helicobacter felis sonicate*, induced IL-10^+ B cells. IL-10^+ B cells were separated from IL-10^- B cells using Regulatory B Cell Isolation Kit. Afterwards, $CD4^+$ T cells were placed in co-cultures with Hact-B cell subsets for 24 hours. Flow cytometry results showed that 20% and 15% of $CD4^+$ T cells co-cultured with IL-10^- B and IL-10^+ B cells respectively, were differentiated to Tr1-like cells. Regarding the IL-10 ELISA results, co-culture of IL-10^+ Breg and $CD4^+$ T cells as well as co-culture of IL-10^- B and $CD4^+$ T cells secrete twice IL-10 compared to only T cells group. Furthermore, according to the IL-17A ELISA results, T cells co-cultured with IL-10^- B cells secreted almost 2,5 fold more IL-17A cytokine when compared to T cells co-cultured with IL-10^+ B cells. Gene expression analysis of IL-17A and RORγt also revealed that IL-10^- B cells induce T cells to express IL-17A and RORγt almost 1.2 fold higher than $IL10^+$ B cells. Overall, it is suggested that Hact-IL-10^+ Breg and IL-10^- B cells both have an effect on the differentiation of $CD4^+$ T cells into Tr1-like cells, while IL-10^- B cells are suggested to play a role in Th17 differentiation.

Keywords: Helicobacter felis; $CD4^+$ T cells; IL-10.

[P-0097]

[Immunity to Infection: New Trends and Developments]

The effect of {*Helicobacter felis*} on bone marrow derived macrophage polarization

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Macrophages exist as heterogeneous and plastic groups, consisting of M1 (pro-inflammatory) and M2 (anti-inflammatory) phenotypes. Moreover, based on their cytokine expression profiles M2 macrophages can be further divided into subsets: M2a, M2b, and M2c. Depending on the conditions, M1 and M2 phenotypes polarize to one another. Since, there is no information regarding the effects of *Helicobacter felis (H. felis)* on macrophage polarization; we aim to investigate the effect of *H. felis* on the polarization of bone marrow derived macrophages (BM-derived macrophages) to either M1 or M2 subtypes. We examined the macrophage polarization by utilizing cell-type specific surface markers of M1 and M2 type macrophages (CD11c and CD206, respectively). Furthermore, different secretion levels of M1 type specific cytokines (IL-12, TNF-α, and IL-1β), M1 type specific NO production, M2 type specific cytokine (IL-10) and also, M1 and M2b type specific cytokine (IL-6) were assessed. Differentiation of BM- derived macrophages was performed using M-CSF. These differentiation levels of BM-derived macrophages were examined using macrophage-specific CD11b-FITC and F4/80-APC antibodies, and analyzed with flow cytometer. 90% of BM cells were differentiated to BM-derived macrophages. Afterwards, BM-derived macrophages were treated with either lipopolysaccharide (LPS) or *H. felis* sonicate for 24 hours. Following the treatment, BM-derived macrophages were stained with M1 type specific antibody anti-CD11c and M2 type specific antibody anti-CD206, and analyzed with flow cytometer. Furthermore, M1 type specific cytokines (IL-12, TNF-α, and IL-1β) and M2 type specific cytokine (IL-10) were assessed by ELISA tests. M1 type specific NO levels were analyzed using nitrite determining Griess reagent protocol. Also, M1 and M2b type specific cytokine (IL-6) levels were determined by real-time PCR. As a result, LPS treated BM-derived macrophages expressed CD11c and secreted high levels of IL-12, TNF-α, IL-1β, IL-6 and also, produced NO; therefore, they were classified as M1 type macrophages. On the other hand, *H. felis* sonicate treated BM-derived macrophages expressed CD206 and secreted IL-10, IL-12, TNF-α, IL-1β and IL-6; however, did not produce NO. Overall, our study shows for the first time that *H. felis* sonicate treatment of BM- derived macrophages leads to polarization of both M2b phenotype with the high anti-inflammatory IL-10 production along with TNF-α, IL-1β, and IL-6 production, and M1 phenotype with the high pro-inflammatory IL-12 production.

Keywords: *{Helicobacter felis}*; bone marrow derived macrophages; macrophage polarization.

[P-0098]

[Immunity to Infection: New Trends and Developments]

The investigation of adenovirus-36 and adipokines such as IL-6, leptin, adiponectin in males with gynecomastia

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Background: In gynecomastia which is characterized as an excessive growth of the breasts in men, it is indicated pathophysiologically that there is an increase in estrogens and decrease in androgens. Ad-36 infection may lead to an increase in regional fat deposition that may consecutively lead to an increased production of estrogens. The interaction of various adipokines with each other secreted from the adipose tissue during a Ad-36 infection involvement may also cause gynecomastia. Following the hypothesis that Ad-36 causing regional fat deposition may also play a role in the disease we investigated the association between Ad-36 and gynecomastia in this study which we previously presented the preliminary data. In this continuation study, we aimed to investigate the status of adipokines (such as IL-6) that can be present at various levels in the presence of Ad-36.

Materials & Method: This study was planned cross-sectional and case-control based study. Thirty-three adult males with gynecomastia (PG) and 15 adult males with anatomic disorders without gynecomastia pathology as patient control group (PCG) were included in this study. Breast reduction samples obtained by the lipoaspiration/subcutaneous mastectomy method, were studied by PCR methods. Simultaneously, Ad-36 antibodies was detected by serum neutralization assay (SNA). Besides, serum IL-6, leptin and adiponectin levels were monitored using ELISA method.

Results: We couldn't detect Ad-36 DNA in breast reduction samples; however, we detected significantly higher Ad-36 antibody levels in the PG compared to the PCG ($p<0.05$). Mean BMI and leptin levels were higher in the Ad-36 positive group, while adiponectin levels were found to be lower in the Ad-36 positive group. Although no statistically significant difference was found IL-6, cholesterol and triglyceride levels between Ad-36 positive and Ad-36 negative group ($p>0.05$), serum leptin and adiponectin levels were found significantly difference between Ad-36 positive and Ad-36 negative group ($p<0.05$).

Conclusion: In this study which was the first in international level, Ad-36 antibodies were detected statistically significant in adults with gynecomastia and our IL-6 results were not detected statistically significant in this association. There is a need for extended serial and particularly cohort based studies in order to have a clear understanding of the Ad-36-gynecomastia relationship.

Keywords: Gynecomastia; adenovirus 36; serum neutralization test; leptin; adiponectin; IL-6.

[P-0100]

[Immunity to Infection: New Trends and Developments]

Adenovirus type 36 seropositivity and levels of leptin, adiponectin, IL-6 in obese children

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Background: Obesity is the most important public health issue these days and infectious agents -especially Adenovirus 36 (Ad-36) - have recently emerged as a possible contributor to the current obesity epidemic. The number of studies about the alleged obesity pathogenesis that developed by adipose tissue Ad-36 trigger is increasing in both the child and adult. The aim of this study was to investigate the prevalence of Ad-36 antibodies in obese children and also investigate the relationship between ˝Ad-36-obesity˝ with the role of serum leptin, adiponectin, IL-6 and lipid levels.

Materials & Method: In this cross-sectional and case-control based study, 71 obese children (BMI % ≥95) and 69 non-obese children (BMI % ≤80) were included in this study as patient group and healthy control group, respectively (age range: 7-17). Simultaneously, the peresence of Ad-36 antibodies and serum adipokine levels were assessed by serum neutralization assay (SNA) and ELISA, respectively.

Results: Presence of Ad-36 antibody was detected in 9 (12.69%) of 71 patients, 1 (1.44%) of 69 healthy control case by SNA and was statistically significant ($p<0.05$). Although serum LDL, total cholesterol, triglycerides, IL-6 and leptin levels were found significantly higher, adiponectin level was found paradoxically low in the obese group. While a significant difference haven't been detected for serum lipids and leptin, adiponectin levels were found significantly lower in adenovirus antibody positive cases of the obese group ($p<0.05$). Ad-36 have been determined as a risk factor with the value OR= 2.057 in the multivariate logistic regression analysis which not indicated a significant difference statistically between groups.

Conclusion: These results demonstrated that Adv-36 presence might has a role in the relationship of children Ad36- obesity etiopathogenesis. Our adipokine results of obese children also support this relationship. However, we believe especially cohort- based studies with larger series are needed to provide clearer results for the relationship.

Keywords: Adenovirus 36; serum neutralization test; children; leptin; adiponectin.

[P-0101]

[Inflammation and Diseases]

Evaluation of hematological parameters in Hashimoto's thyroiditis

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In this study, we want to evaluate the hematological parameters in Hashimoto's thyroiditis (HT) because of the effects of lymphocytes and platelets on the pathophysiology of HT are still unknown.

This study included 54 patients with HT and 20 control subjects. Among the HT patients, three subgroups were formed according to thyroid function: overt (n=10), subclinical (n=20), and euthyroid (normally functioning thyroid; n=24).

Demographics were similar between the patient with HT and control groups. Body mass index (BMI) was higher in the patient with HT than in the control group. The C reactive protein (CRP) level was higher in patients than controls (p=0.051). The thyroid stimulating hormone (TSH) level was higher and the mean free thyroxine level lower in the patient group than in the control group (p<0.01). The neutrophil lymphocyte ratio (NLR) and platelet lymphocyte ratio (PLR) were significantly different in one subgroup of HT patients relative to healthy subjects (p<0.01). However, we did not find any statistical differences in the mean platelet volume (MPV) among the three subgroups (p=0.701). A positive correlation was found among the NLR, anti-thyroglobulin (TG) antibodies, and anti-thyroid peroxidase (TPO) antibodies (p<0.05), although there was a negative correlation between the PLR, TSH, anti-TPO, and anti-TG (p<0.01).

This preliminary study show that a single marker or panel of biomarkers is not a consistent indicator of HT, but NLR combined with PLR testing may offer a more reliable diagnosis.

Keywords: Hashimoto's thyroiditis; hematological parameters; neutrophil lymphocyte ratio.

[P-0102]

[Inflammation and Diseases]

Endocan and asymmetric dimethylarginine levels in patients with hypothyroidism

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Endocan, also known as endothelial cell-specific molecule, is a new biomarker for ED in various diseases, especially atherosclerosis. The asymmetric dimethylarginine is another cardiovascular disease specific marker. This study aimed to determine the relationships between serum endocan and asymmetric dimethylarginine (ADMA) level in patients with hypothyroidism.

Twenty-one patients with hypothyroidism and positive thyroid antibodies (anti-thyroid peroxidase [anti-TPO] antibody and anti-thyroglobulin [anti-TG] antibody) were included in the study. Eleven age and sex matched euthyroid healthy subjects with negative anti-TPO antibody were chosen as the control group.

Anthropometric measurements, thyroid function test results, serum lipid parameters, endocan, and ADMA levels were measured at the time of diagnosis. The endocan and ADMA levels were assessed using an ELISA kit according to the manufacturer's instructions.

The mean serum endocan levels in the patient group and control group were 0.81±0.15 ng/mL and 0.63±0.09 ng/mL, respectively (p=0.024). The mean serum ADMA level was significantly higher in the patient group compared to control group (0.97±0.19 mmol/L and 0.46±0.20 mmol/L, p<0.001).

This study is the first documentation of endocan and ADMA as biomarker in patients with hypothyroidism. These markers were shown statistically significant correlation. Therefore, prospective studies should be planed about endocan and ADMA end-points levels because of there is necessary to confirm these findings.

Keywords: Hypothyroidism; endocan; asymmetric dimethylarginine.

[P-0103]

[Inflammation and Diseases]

Resistance to polyene antifungals that used prophylactic purposes in immune deficiency, chemotherapy and transplantation

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Optimal management of fungal infections in patients with cancer, transplant recipients, immune deficiency and AIDS are very troublesome process. These infections can be life-threatening, especially when they spread throughout the body. Nystatin (N) is sometimes given as a routine preventive measure or as treatment in these patients. The oldest of the drug Amphotericin B (AMB) often used intravenously for serious systemic fungal infections.

In this study, we evaluated the trends in C. albicans polyene resistance over the past 15 years. The study was planned and conducted in accordance with the declaration of PRISMA and involved a literature search, determination of inclusion criteria for, and evaluation of, articles, data collection and statistical analysis. Following application of appropriate criteria, 20 studies published between 2000 and 2015 was performed.

In our study, mean rates of resistance to AMB and N were 2.51±5.85% and 12.35±18.58, respectively. The mean rates of resistance to antifungals differed significantly both with time and geographically. Using a random-effects model, the event rates of resistance to AMB and N were 1.6% and 8.5%, respectively.

In our study, the development of resistance to polyene in our country changed over time and differs geographically. Therefore, the use of polyene may be recommended used in combination with other antifungals in patients with immune deficiency, chemotherapy and transplantation.

Keywords: Immune deficiency; chemotherapy; transplantation; prophylactic therapy.

[P-0104]

[Inflammation and Diseases]

Is there any concern between very high white blood cell count and procalcitonin level in diagnosis?

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A diagnostic test is considered reliable if the same results are produced repeatedly, if it were to be carried out again. Procalcitonin (PCT) is widely used as a suitable biochemical marker to differentiate sepsis from non-infectious systemic inflammatory response. Aims of the current study were to determine whether the PCT may be suggested a "specific target value" to be maintained during the course of bacterial infection diagnosis.

PCT was measured using the enzyme-linked fluorescent immunoassay (ELFA) on the miniVIDAS instrument and fluorescence immunoassay on the i-CHROMA according to the manufacturer's recommendations. The PCT assays with mini-VIDAS and i-CHROMA have a measuring range from 0.05 to 200 ng/mL and 0.25 to 100 ng/mL respectively. Cut-off value was taken 0.5 ng/mL for both system.

There was statistical significant difference between i-CHROMA and mini VIDAS PCT positivity rate (p=0.007) which were calculated 47.4% (18/38) and 65.8% (26/38) respectively. The mean i-CHROMA and mini VIDAS PCT values were found 3.6±7.8 ng/mL, and 4.5±8.8 respectively. i-CHROMA did not give gray zone positivity, the mini VIDAS gave gray zone positivity for 8 (21.1%) patient who has higher white blood cell count (WBC) (>30 000 /mL).

We can conclude that the change in mini VIDAS PCT level was very small but this inconsistency should be debated.

Keywords: Procalcitonin; sepsis; inflammatory response.

[P-0107]

[Inflammation and Diseases]

Evaluation of the Soluble urokinase plasminogen activator receptor serum levels in patients with Parkinson's disease

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Parkinson's disease (PD) is the most widespread neurological disorders characterized by degeneration of cognitive and/or motor functions. Numerous evidences have established the role of neuroinflammation in the PD pathology. Soluble urokinase plasminogen activator receptor (suPAR) is a highly sensitive marker that reflects increased inflammation and is positively correlated with pro inflammatory biomarkers. In this pilot study, suPAR serum levels were investigated in patients with PD.

Twenty patients with PD were recruited from outpatient clinics of Neurology department. Diagnosis of PD was based on United Kingdom PD Society Brain Bank Diagnostic Criteria. Secondary causes or other neurodegenerative diseases were excluded. The serum suPAR concentration was quantified by enzyme-linked immunosorbent assays (ELISA). This is an experimental study. In this study, *in vitro* samples were investigated; any samples were not taken from patient.

The patients with PD were done with two sections according to disease duration, early stage of PD (ESPD, n=10) and late stage of PD (LSPD, n=10). Relating to disease duration, suPAR levels were found significantly different. In LSPD group was found high suPAR level (2.7±1.1 ng/mL) than ESPD group (1.9±0.6 ng/mL) (p=0.028). The suPAR serum levels did show statistical significant differences in patient conditions in terms of clinical features such as dyskinesia, unified PD rating scale (UPDRS), Hohn-Yahr Staging Scale (HY) and MMSE status.

The uPAR is expressed on a number of different cells including monocytes, macrophages and neutrophils.[Serum levels of suPAR have been shown to be a possible indicator in patients with various diseases, such as cancer, autoimmune diseases, tuberculosis and sepsis. Furthermore, high levels of suPAR are accused with increased risk of mortality and reflect disease severity. The use of suPAR may be recommended for differentiation of disease severity in patients with PD. But prospective studies should be planed about suPAR end-point levels for ESPD and LSPD.

Keywords: Parkinson's disease; neuroinflammation; suPAR.

[P-0108]

[Immunodeficiencies: Emerging Molecular & Cellular Therapies. Remembering Prof. Isıl BARLAN]

SLCA29A3 gene defect in a patient with autoinflammatory disease and pure red blood cell aplasia: H syndrome

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H Syndrome (HS) is an autosomal recessive (AR) genodermatosis associated with systemic findings caused by SLCA29A3 gene defect. SLC29A3 encodes a 475 aa protein named 'human intracellular equilibrative nucleoside transporter 3' which is localized in late endosomes/lysosomes. This protein is thought to have role in transport of nucleotides/nucleobases/nucleotide analogs from lysosomal membrane and inner mitochondrial membrane to cytoplasm, maintaining a cytoplasmic pool for several cellular pathways. Here, a male patient having pure red cell aplasia (PRCA), articular deformities is presented. He was given the diagnosis of H Syndrome at the age of 26 years with whole-exome-analysis.

A 22-year-old male patient, who was the fourth child of first-degree cousin parents referred to Immunology department for possible primary immunodeficiency (PID). After the age of 1.5 years, progressive articular deformities in small joints developed, and he started to take weekly erythrocyte transfusion for severe anemia due to PRCA. He had been operated for foot deformities. He had a history of a male sibling death. His elder brother had similar clinical findings, died at the age of 12 with renal failure. On physical examination he had vitiligo, cutaneous fungal skin lesions on feet, severe sensorineural hearing loss, had severe contractures/deformities in hands and feet. He had severe anemia. Immunologic test results showed lymphopenia, mildly low lymphocyte proliferation compared with healthy control. Acute phase reactants were elevated, and immunosuppressive therapies given before were not sufficient to control the progression of disease. In the follow-up, anti-GAD, anti-islet-cell, anti-TPO antibodies were found to be positive, given the diagnosis of diabetes mellitus. Biopsy taken from mesenteric lymphadenopathy was compatible with fungal infection. After intravenous immunoglobulin (IVIG) therapy was started, hematologic findings resolved, he had no further erythrocyte transfusion need, and progression of articular deformities stopped. As there is a consanguineous pedigree, sibling death history, whole-exome-analysis was performed for possible PID associated with autoimmunity. SLCA29A3 gene defect (c.1394 C>G; p.Ser465) was found. Now, he is fine, on monthly intravenous immunoglobulin therapy and low dose steroids (0.15 mg/kg).

Several clinical findings; hyperpigmentation, hypertrichosis, hepatosplenomegaly, deafness, heart anomalies, hypogonadism, short stature, hyperglycemia/diabetes mellitus, hallux valgus/flexion contractures may be present in HS. Hyperpigmentation, hypertricosis defined in most of HS patients were not present in present patient. Vitiligo was not reported in HS before. IVIG therapy was useful with possible immunomodulatory effects in disease progression and resolution of hematologic findings. Whole-exome-analysis was useful to reveal the genetic defect in this very rare AR syndrome.

Keywords: Autoinflammatory disease; pure red blood cell aplasia; immunodeficiency.

[P-0109]

[Novel Discoveries in the Innate Immune System]

Differential immunomodulatory activities of pathogenic and commensal derived RNAs

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Bacterial RNAs are recognized by various types of immune sensors. Depending on whether the bacterial RNA is originating from commensal or pathogenic bacteria, recognition of this PAMP shows significant difference including cell types, sensors, pathways and type of secreted cytokines. Here, we aimed to investigate the differential activation mediated by RNAs. For this, total RNA as well as ribosomal RNAs (5s, 16s and 23s) are isolated from twobona fide commensal bacteria, Lactobacillus salivarious and Lactobacillus fermentum and two strict pathogens, Listeria monocytogenes, and *Streptococcus pyogenes*. Bacterial RNA isolated from pathogens, but not from commensals, triggered NF-κB and AP-1 activation in HEK-Blue cells separately expressing TLR3 and TLR7. Furthermore RNAs of pathogens induced significantly stronger pro-inflammatory cytokines from human peripheral blood mononuclear cells (hPBMCs) compared to RNAs derived from commensal bacteria. Conversely, pathogenic but not commensal RNA failed to induce detectable type I IFN production. Type I IFN response can be detected after both total RNAs and rRNA subunits derived from commensal bacteria are delivered to cytosol with transfection reagent Lipofectamine 2000. This suggests that commensal bacterial RNA might possess structural features recognized by cytosolic RNA sensors like RIG-I and MDA-5. To identify the source of type I IFN response, we depleted plasmacytoid dendritic cells (pDCs) from hPBMC since they are the major producers of type I IFNs. Depletion of pDCs from hPBMCs significantly diminished IFN-α secretion in response to both total and rRNAs. Furthermore after the stimulation with commensal derived total RNA and rRNA subunits, IRF activation in THP1-Blue ISG cells was observed in a dose dependent manner. In conclusion, our data implicated that RNA from commensals and pathogens are recognized differentially by immune cells (endosomal TLR vs cyrosolic RNA sensors, respectively) and induce divergent pathways leading to a diverse immune response suitable for different therapeutic applications.

Keywords: Bacterial RNA; commensal; RIG-I/MDA-5; type-I interferon.

[P-0112]

[Immunity to Infection: New Trends and Developments]

Development of {L. tropica}-derived exosome-based preventive vaccine against {L. tropica} induced cutaneous leishmaniasis

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Leishmaniasis is an infectious disease caused by Leishmania protozoa transmitted to mammalian hosts by infected sand flies. The World Health Organization considers leishmaniasis to be the second most serious tropical parasitic diseases after malaria. The absence of a good protective vaccine against leishmaniasis limits prevention options and enforces the use of highly toxic pentavalent antimonials for treatment. Herein, we aimed to develop a prototype preventive vaccine against L. tropica cutaneous infection in mice. For vaccine development, first purified, Leishmania antigen-rich small vesicles (exosomes) secreted from L. tropica amastigotes. Following their physical and biochemical characterization, the exosomes were combined with several different vaccine adjuvants or their combinations, including, cyclic-di-GMP, different classes of CpG ODNs, CpG nanorings and their immunoprotective activity were tested in a mouse model of cutaneous Leishmaniasis. To monitor disease progression, lesion sizes were monitored for 10 months and parasite burdens in each group was predicted by real time PCR. Leishmania soluble antigen-specific serum IgG1 and IgG2a antibody titers were assessed by ELISA. Our results show that Th1 type vaccine adjuvanted exosomes (particularly the combination of CpG ODN and cyclic-di-GMP) suppressed parasite burden, increased serum anti leishmania IgG2a antibody titers and enhanced the protective ability of the exosomes. In conclusion, the exosome based vaccine approach tested herein plus the proposed adjuvants constitute a novel approach in design of vaccines against neglected parasitic diseases.

Keywords: {L.tropica}; cutaneous leishmaniasis; exosome; vaccine.

[P-0117]

[Immunity to Infection: New Trends and Developments]

Investigation of humoral immune response against nucleoprotein (NP) of crimean congo hemorrhagic fever virus (CCHFV) in mice

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Crimean Congo Hemorrhagic Fever is a severe tick-borne hemorrhagic disease caused by CCHFV, belonging to "Nairovirus" genus in the "Bunyaviridae" family. Crimean Congo Hemorrhagic Fever Virus is one the most wide spread tick-borne virus affecting number of countries in Asia, Africa, Middle East and Europe including Turkey and reported to be the second most medically important virus following Dengue virus. Mortality rates of disease change between 3 to 30% according to infecting strain. Information related to the pathogenesis and biology of the virus is limited due to the fact that the virus is considered to be a level 4 pathogen which requires higly specialized laboratories for investigations. Crimean Congo Hemorrhagic Fever Virus is a negative stranded RNA virus with three segments named Small (S), Medium (M) and Large (L) segments encoding nucleoprotein (NP), surface proteins (Gn and Gc) and RNA dependent RNA polymerase (RdRp), respectively. In bunyaviruses, Np is the most abundant viral protein produced. The role played by NP during the viral infection as well as in the immune response against the virus mounted by the host are under scrutiny. Here, we investigated the humoral immunity raised against the whole NP (482 amino acids) and two fragments of Np (127 amino acid long N-terminal and 195 amino acid long C-terminal fragments) in mice. After bacterial expression of the proteins, Balb/c mice were immunized with different doses of the proteins and antibody titers determined by ELISA. Also, anti NP antibody responses in CCHFV positive human sera were screened with bacterially expressed NP. The results indicate that NP is capable of inducing antibody response in the natural infection as well as in the experimental model which warrants further studies to elucidate the precise role of this immune response in CCHFV infection process.

Keywords: CCHFV; NP; humoral immune response.

[P-0119]

[Inflammation and Diseases]

The relationship of netrin-1 expression with macrophage infiltration and polarization in human epicardial adipose tissue and severity of coronary artery disease

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Introduction: Epicardial adipose tissue is the visceral adipose tissue layer located between the myocardium and serous pericardium. Inflammatory activity, and adipokines and cytokines originating from the epicardial adipose tissue are thought to have a role in coronary artery disease pathogenesis. Factors mediating the enhanced inflammatory activity in the epicardial adipose tissue in patients with coronary artery disease is a topic still under debate. This study aims to investigate the relationship between macrophage infiltration and polarization in the epicardial adipose tissue and Netrin-1 gene expression.

Methods: Macrophage infiltration and polarization have been examined by immunohistochemical methods. Gene expression levels of Netrin-1, Unc5b and cytokines related with macrophage subtypes (IL-12 and IL-18) have been determined by qPCR in subcutaneous and epicardial adipose tissue. Samples obtained from patients with coronary artery disease undergoing coronary artery by-pass grafting. Patients undergoing non-coronary cardiac surgery were assigned to the control group and the same sample acquisition and examination protocol was applied.

Results: Increased macrophage infiltration and pro-inflammatory shift in macrophage polarization status in favor of M1 macrophages were observed in epicardial adipose tissue of patients with coronary artery disease when compared to the control group. Furthermore, in patients with coronary artery disease, Netrin-1, Unc-5b and IL-18 gene expression levels were significantly elevated in epicardial adipose tissue (relative to the gene expression levels in subcutaneous adipose tissue) when compared to the control group. Netrin-1 gene expression in epicardial adipose tissue in patients with coronary artery disease was also significantly correlated to the parameters of macrophage infiltration and polarization.

Conclusion: The link between enhanced Netrin-1 expression in epicardial adipose tissue and macrophage infiltration and polarization in patients with coronary artery disease has been elucidated for the first time in this study.

Keywords: Netrin-1; epicardial adipose tissue; macrophage polarization; coronary artery disease.

[P-0121]

[Cellular and Molecular Targets in Rheumatoid Arthritis and Autoimmune/Autoinflammatory Diseases]

HSP60 as an autoantigen in obesity

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Although the association of a chronic low-grade inflammation with obesity has long been appreciated, its molecular basis is yet to be defined. The proven involvement of adaptive immunity, coupled with a phenotypic switch from autoimmune suppressive tolerogenic Treg to pro-inflammatory CD4+ Th1 and CD8+ T cells, during progression of obesity necessitates the presence of a triggering antigen as an activator of T and B cells. Not surprisingly in this context, it is found that visceral adipose tissue-specific T cells show severely biased T cell receptor Vα repertoires in diet induced obese mice (Winer et al. 2009), implying an antigen-specific clonal expansion of T cells during obesity.

HSP60 is an evolutionary conserved mitochondrial chaperonin that assists the correct folding of other mitochondrial proteins. However, its occurrence is not restricted to mitochondria and it can be located in the cytosol or exposed on the cell membrane also. An increase in cell membrane HSP60, which may be accompanied by HSP60 release into circulation, is especially considered a signal of autoimmunity. HSP60 has been associated with a broad range of diseases so far, particularly those with an autoimmune component. More recently, HSP60 is also linked to obesity as a mediator of adipose tissue inflammation and insulin resistance. Moreover, circulating HSP60 levels are found to be higher in obese individuals than lean controls (Märker et al. 2012).

We observed an adaptive immune response against HSP60 at both T cell and B cell (antibody) levels during continuous high fat feeding of C57bl6 mice. Hence HSP60 appears to be one of the mystery auto-antigens triggering the early T and B cell responses during obesity. Furthermore, we attempted a peptide therapy in a dose escalation protocol aiming to down-regulate the inflammatory related adverse effects of obesity by achieving tolerance in T cell populations and suppressing the pathogenic antibody response. Treatment with a mixture of three proven immunomodulatory HSP60 peptides did not reduce weight but completely reversed the increase in VLDL/LDL levels and partially reversed the glucose intolerance in obese mice, which encourages further research to improve peptide therapy.

Keywords: Autoimmunity; inflammation; obesity; HSP60; autoantigen; immunotherapy.

[P-0122]

[Tumor Immunology & Molecular Markers]

The anti-tumor activity of natural killer cell-derived exosomes

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Cells can communicate through a variety of (macro)molecules either locally or distally. Those macromolecules can function in cell-junctions proximally or by freely circulating in the blood such as hormones or free circulating nucleic acids in a longer range. Exosomes, which are membranous nanovesicular structures with a diameter ranging between 30-130 nm, are another emerging means of distal cellular communication. Their cargo are composed of a myriad of different molecules including nucleic acids (e.g. miRNA, mRNA, DNA, lncRNA) and proteins either internally (e.g. Tsg101, Alix) or as a part of their enclosing membranous structure (e.g. CD9, CD63, CD81). Exosome content varies depending on the cellular origin as well as the current state of the cell (i.e. type of stimuli, stage of cell cycle, healthy vs cancerous etc.). Exosomes are gaining an accelerating interest as biomarkers for various cancer types as well as drug delivery systems for their inherent cellular targeting mechanism causing an immune response *in vivo*.

Here we describe the purification of exosomes from a natural killer (NK) cell line, NK92, using a fast protein liquid chromatography (FPLC) system with a size exclusion chromatography (SEC) column. Additionally, our data supports that the NK-derived exosome yield depends on the cell culture growth conditions (i.e. cell culture media, cytokine stimuli). The physical characterization of purified exosome fractions were performed using nanoparticle tracking analysis (NTA) in order to determine the concentration and size distribution of exosomes. Further biochemical

characterization was done using current consensus exosomal markers such as CD63, CD81, Alix and Tsg101 by western blotting. The functional characterization of the purified exosomes was performed on a cancer cell line, K562, for cytotoxic effect. K562 is a human leukemia cell line which is easily killed by NK cells since they lack the major histocompatibility complex required to inhibit NK activity. Finally, for labeling of NK-derived exosomes we made use of a bicistronic lentiviral vector which has a tdTomato reporter and demonstrated the localization of three different exosomal markers- CD9, CD63, CD81- using fluorescent microscopy.

* This research is supported by the Scientific and Technological Research Council of Turkey (TUBITAK) grant 114Z343.

Keywords: Exosome; biomarker discovery; drug delivery; NK92; natural killer cells.

[P-0123]

[Immunity to Infection: New Trends and Developments]

The impact of *Pseudomonas aeruginosa* colonization on the functional and cytokine secretion capacity of neutrophils in cystic fibrosis

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Objectives: Cystic fibrosis (CF) is the most common and lethal genetic disease of Caucasians that affects children and young adults. Beside their likelihood to be infected by ordinary pathogens, most of CF patients' respiratory system is chronically colonized with the opportunistic *Pseudomonas (P) aeruginosa*. This chronic infection negatively affects the clinical outcome in these patients, through a neutrophil-guided chronic inflammation and impaired host defenses. In this study; to understand the innate immune functions of patients with CF who were chronically colonized by *P. aeruginosa*, we investigated survival rate, functional analysis and adhesion molecule expression of neutrophils. Additionally, we investigated CAP-18 and pro-inflammatory cytokine secretion of neutrophils under bacteria and LPS stimulation.

Methods: We allocated patients with CF into two groups according to sputum culture results for *P. aeruginosa*: Positive (P+) (n=8, mean age 10.1±3.2 years) and negative (P-) (n=8, mean age 11±2.8 years) and then compared them with age- and sex-matched healthy controls (n=8, mean age 11.1±4.6 years). Surface expression of adhesion molecules on neutrophils $CD11a^+CD18^+$, $CD11b^+CD18^+$, $CD11c^+CD18^+$, CXCR2 as well as neutrophil apoptosis were analyzed using flow cytometry. CAP-18, IL-8, and TNF-α secretion of $CD16^+CD66b^+$/$CD16^+CD66b^-$ neutrophils were also analyzed using flow cytometry after *P. aeruginosa* and LPS stimulation for 18 h at 37°C.

Results: Higher levels of CXCR2, $CD11a^+CD18^+$, $CD11b^+CD18^+$ were observed on the neutrophils of P- patients compared with the other groups. Patients with CF had higher capacity of phagocytosis of *E. Coli* than the healthy controls. The P+ group had higher oxidative burst levels and *P. aeruginosa* phagocytosis ability then the others. The P- group showed a higher *P. aeruginosa* phagocytosis capacity compared with the healthy controls. Early and late apoptotic neutrophils of P- and P+ patients were increased than the healthy controls. In unstimulated condition, higher levels of $CD66b^+CD16^+$, $CD16^+TNF\text{-}\alpha^+$, $CD16^+CD66b^+TNF\text{-}\alpha^+$ and $CD16^+IL8^+$ were determined on the neutrophils of patients with CF, whereas bacterial stimulation significantly decreased secretion of CAP-18 on both $CD16^+$ and $CD16^+CD66b^+$ neutrophils compared with healthy subjects. LPS stimulation also caused a decrease in CAP-18 levels on $CD16^+CD66b^+$ cells but only in the P+ group, compared with controls.

Conclusion: Patients with CF and *P. aeruginosa* colonization showed higher neutrophil activation beside a decrease in CAP-18 levels. These findings affirm the inadequate neutrophil defense toward the pathogen with a destructive effect on the airways, which results in severely reduced lung function.

Keywords: Neutrophiles; cystic fibrosis; cytokines; CAP-18.

[P-0124]

[Inflammation and Diseases]

A case with TACI mutation

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Common variable immune deficiency (CVID) is an assorted group of primary antibody failure syndromes that clinically manifests with antibody deficiency, infection susceptibility, and autoimmunity. Underlying genetic causes are only known in less than 15% of patients and encompass mutations in the genes encoding for ICOS, TACI, BAFF-R, CD19, CD20, CD81 and MSH5. Transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) is the most frequently mutated gene among CVID patients. The C104R mutation in TACI is the most frequent mutation identified in patients with CVID.

We report a 17 month-old female born from non-consanguineous parents and presented with a history of recurrent upper respiratory tract infections and weight loss. She was at 3. percentile for weight and 10-25. percentile for height with normal mental status. No abnormal physical finding was found. There was tonsillar tissue without lymphadenomegaly. She did not have any skin lesions. Laboratory findings were normal except specific antibody deficiency (anti B: 1/4) and hypogammaglobinemia [IgG: 236 mg/dL (365-1200), IgA: <5.6 mg/dL (11.5-94.3), IgM: <19.1 mg/dL (25.6-201)]. Whole TACI gene sequencing revealed homozygous missense variant (p.Cys104Arg).

Immune deficiency should be considered in patients who have frequent infections. Patients should be investigated about TACI gene mutations especially with presentation of autoimmunity and lymphoproliferation.

Keywords: İmmun deficiency; CVID; TACI mutation.

[P-0125]

[Tumor Immunology & Molecular Markers]

The role of natural killer cells in B-cell acute lymphoblastic leukemia

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Leukemia is a hematological disease which is seen in blood and bone marrow. Symptoms may include bleeding and bruising problems, feeling very tired, and an increased risk of infections. Leukemia is subdivided into a variety of large groups. One of the subgroups of it is Acute Lymphoblastic Leukemia (ALL); lymphocyte progenitor cells' maturation process break down at bone marrow so that impaired cells' proliferation become uncontrollable. This abounded proliferation cause ALL. According to source of progenitor blast cells, ALL classified as B-ALL and T-ALL. Although there is no clinical evidence of leukemia disease relapse like leukemia. In that times Minimal Residual Disease (MRD) tests are applied. In researches there are strong connection between MRD level and respond to treatment.

Bone marrow derived Natural Killer (NK) cells are large granular lymphocytes playing a role in the innate immunity. NK cells exert potent spontaneous cytolytic activity against tumor cells and compose 15% of the peripheral lymphocytes. These cells are present in tissues such as liver, peritoneal cavity and placenta. It known that the expression of MHC-I molecules on leukemia cells' surfaces is low. If there is no MHC-I expression, tumor cells are recognized by NK cells' surface receptors, especially NKG2D activator receptors, and killed.

In this study, we aimed to investigate NK cells' immunological role in B-ALL patients' peripheral blood and bone marrow. Therefore CD16 and CD56 expressions and cytoplasmic IFN-γ, IL-4, IL-10 secretions of NK cells isolated from blood and bone marrow were assessed by flow cytometry after stimulation by PMA and ionomycin for 5 hours.

The CD16+ expression of unstimulated peripheral blood NK cells are increased than unstimulated NK cells of bone marrow. On the contrary cytoplasmic IFN-γ cytokine levels of CD16+ NK cells from bone marrow are increased than blood's CD16+ NK cells. So that NK cell mediated responses are increasing in bone marrow. However, when B-ALL patients and healthy controls are compared, it shows B-ALL patients have lower amount of NK cells. In that case B-ALL NK cell mediated responses are not sufficient.

In peripheral blood unstimulated CD16+ and CD56+ NK cells have low level of IL-4 expressions. On the other hand proliferated NK cells with IL-2 have high level of IL-4 expression. When bone marrow's NK cells evaluated in itself, unstimulated CD56+ cells have low level of IL-10 expression. In addition, there is no difference of IL-10 levels in PMA stimulated NK cells.

Keywords: Immunology; NK cells; cytokines.

[P-0126]

[Emerging Issues in Adaptive Immune System]

Evaluation of the effect of storage temperature on D-dimer stability, using two different techniques

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Our study designed to assess the stability of D-dimer levels and storage conditions, using two different techniques. This is the first study to investigate the short-term storage stability of D-dimers in clinical citrated plasma samples containing a range of D-dimer concentrations.

This study was performed with 60 samples that were collected randomly patients with suspected thromboembolism. Plasma samples were incubated for eight different time intervals (0 (i.e., fresh samples), 4, 24, 48, 72, 120, 168, and 240 h) and at three different temperatures (25+2 °C, 4+2 °C, and -20+2 °C) to evaluate the effect of storage conditions on D-dimer stability. Plasma D-dimer concentrations were determined using two different techniques, a fluorescence-based sandwich immunodetection assay (i-CHROMA, Boditech Med Inc., Korea) and an enzyme-linked immunosorbent assay (ELISA) (VIDAS, bioMerieux, France).

D-dimer levels in the plasma samples did not differ after 4 or 24 h compared with 0 h when stored at room temperature. However, longer storage times (48 and 72 h) at room temperature resulted in reduced D-dimer levels compared with 0 h (19.3% and 35.9%, respectively).

In accordance with our study, we suggest D-dimer analysis be performed on plasma samples stored for <48 h at room temperature. Our findings indicate that i-CHROME and VIDAS D-dimer measurement methods have remarkably high sensitivity and are safe first-line tests that can be utilized to rule out pulmonary emboli in outcome studies.

I-CHROME, which is a point of care test, is suggested for samples stored under appropriate conditions. This and future studies that compare different methods and storage conditions and test a number of variables will be necessary for the standardization of D-dimer tests.

Keywords: D-dimer; frozen plasma; fresh plasma; stability; room temperature; multiple freeze-thaw.

[P-0127]

[Cellular and Molecular Targets in Rheumatoid Arthritis and Autoimmune/Autoinflammatory Diseases]

A retrospective evaluation of anti-mitochondrial antibody positivity in a university hospital

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Antimitochondrial antibodies (AMA) are the serologic hallmark for primary biliary cirrhosis. Furthermore several other autoimmune disorders are commonly found in association with chiefly autoimmune hepatitis, autoimmune thyroiditis, Sicca syndrome, Raynaud's disease, systemic sclerosis, CREST syndrome, rheumatoid arthritis and celiac disease. Patients with suspected autoimmune disease were screened for the presence of anti-mitochondrial antibodies using an indirect fluorescent assay (IFA) and/or extractable nuclear antigen (ENA) tests in rat liver and kidney tissues.

A total of 55 AMA M2-positive patients who had been studied between May 2012 and March 2016 in Medical Microbiology Laboratory of Ministry of Health, Sakarya University Education and Research Hospital were included in the study. Samples were screened for reactivity against human epithelial cancer (HEp) 2010 cells, and evaluated using indirect fluorescent microscopy. Furthermore the immunoblot test (ANA profile 3 Euroline; Euroimmun, Germany) was studied at 1/100 dilution, according to the manufacturer recommendations. The immunoblot evaluations were performed by making use of a scanner (Canon ScanLide 100; Canon, Japan) and EurolineScan (Euroimmun, Germany) software.

The largest percentage of AMA positive patients were associated with Gastroenterology clinic (45.4%), Rheumatology clinic (43.6%), Internal Medicine clinic (5.4%) followed by Physical Medicine and Rehabilitation clinic (1.8%), Neurology clinic (1.8%), urology clinic (1.8%). Of the 25 patients from the gastroenterology clinic, 17 had primary biliary cirrhosis, 2 had autoimmune hepatitis, the other 2 had overlap syndrome and 1 had Sjögren's syndrome diagnosis. Of those 24 patients who had been tested in the rheumatology clinic for AMA M2 due to polyarthritis etiology and were found as positive, 3 patients had rheumatoid arthritis, 2 had Sjögren's syndrome, 1 had SLE, and 1 patient had a diagnosis of Behçet's disease.

In this preliminary study, AMA positivity was found compatible with literature. But beyond our knowledge, 3 AMA positive cases could not explain for clinical diagnosis.

Keywords: Indirect immune fluorescence test; extractable nuclear antigen; antimitochondrial antibody; autoimmunity.

[P-0128]

[Tumor Immunology & Molecular Markers]

The effect of THP-1-derived macrophages on helper T cell proliferation and activation

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Introduction and Aim: THP-1 monocytic leukemia cells have been recognized as the most appropriate cell line representing human monocytes. Upon treatment with the protein kinase C (PKC) agonist, phorbol 12-myristate 13-acetate (PMA), these cells differentiate into macrophage-like cells. In this study, the generation of THP-1 macrophages was optimized in order to establish an *in vitro* model to assess macrophage and helper T (Th) cell interaction that leads to activation, differentiation, proliferation and eventually exhaustion.

Methods: THP-1 cell line was cultured in the presence of PMA for different periods (for 24, 48, 72 and 96h). Macrophage differentiation was followed-up with the markers CD11b, CD11c, CD33, HLA-DR, CD14, CD68, CD80, CD86, PD-L1, PD-L2, ICOS-LG, B7-H3 and B7-H4 by using flow cytometry. The morphology of PMA-treated and control THP-1 were determined with Giemsa staining. Immunohistochemistry for CD68 was also performed. Additionally, the effect of PMA treatment on THP-1 cells' proliferative capacity was assessed with a flow cytometric CFSE assay. Then, macrophage-like (PMA-THP-1) cells were co-cultured with monocyte-depleted PBMCs or with purified CD4+ T-cells in the presence of anti-CD3 mAb. CD8 and CD4 T cell proliferation was assessed with eFlour647 assay in the co-cultures established with various ratios of PMA-THP-1 cells. Activation status of Th cells was determined with the expression of markers CD154, CD25, CD69, TIM3, LAG3, HLA-DR and PD-1 by flow cytometry.

Results: Upon 72h of PMA treatment, THP-1 cells became firmly adherent, displayed fine membrane projections, lost proliferation capacity and CD11b, CD11c and CD68 molecules. Expression of CD14, HLA-DR and CD33 was slightly decreased. These cells were capable of inducing T cell proliferation through co-stimulatory actions potentially through CD80 and CD86 molecules. On the other hand, increasing the co-culture ratio, PMA-THP1 macrophages: monocyte-depleted PBMCs, over 0.125:1 did not enhance either CD4+ or CD8+ T cells' proliferation. Moreover, Th cells were identified with exhaustion markers TIM3, PD1 and LAG3 even at the early phases of co-cultures. CD4 co-receptor levels were also diminished in the co-cultures.

Conclusion: Here, THP-1-derived macrophages were generated and an *in vitro* co-culture model was established to monitor T-cell macrophage interactions that might have implications on T cell hypo-responsiveness.

Keywords: PMA; AML; T cell exhaustion.

[P-0129]

[Tumor Immunology & Molecular Markers]

Determination of suppressor and cytotoxic lymphocyte populations and cytotoxic mechanisms in patients with early stage non-small cell lung cancer

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Objectives: Lung cancer is the leading cause of cancer-related death in both men and women worldwide, accounting for 1.2 million new cases annually and the five-year mean survival rate has been reported as 5-10%. Immunity to lung cancer cells is poorly understood. In this study we aimed to analyze the ratio of circulating lymphocytes, suppressor and cytotoxic T cell subsets and cytotoxic mechanisms of natural killer (NK) and CD8+ T cells in order to determine immune scoring.

Methods: The study groups comprised patients with newly-diagnosed non-small cell lung cancer (NSCLC) (n=11, mean age: 68 ± 7.8 years) with T1-3N0M0 NSCLC, none of whom had received preoperative chemotherapy and/or radiotherapy, and healthy subjects (n=25, mean age: 50 ± 7.8 years). Lymphocyte subsets, the prevalence of $CD4^+CD25^+Foxp3^+$, and $CD8^+CD28^-$ regulatory T (Treg) cells were analyzed using flow cytometry. NK cells and subsets of $CD8^+$ T cells (CD8dim and CD8bright) were studied through their cytotoxic capacity. Peripheral blood mononuclear cells that stained with CD107a were incubated with K562 cells and unstimulated (US) conditions with an effector: target ration of 10:1 for 4 hours and CD107a expression was analyzed using flow cytometry.

Results: CD8dim, NK, NKT, $CD4^+CD25^+$, $CD8^+CD16^+$ cells, and $CD8^+CD28^-$ suppressor T cell subsets were found to be increased and percentages of B and $CD3^-HLADR^+$ lymphocytes were detected to be decreased in patients with NSCLC (p=0.012, p=0.000, p=0.002, p=0.000, p=0.031, p=0.008, p=0.033, p=0.002 and p=0.004). Although the cytotoxic activity of NK cells did not differ between the groups, CD107a expression was found

increased in total $CD8^+$ and CD8dim T cells in US conditions and also in total $CD8^+$ T cells after stimulation with tumor cells in the NSCLC group (p=0.046, p=0.044 and p=0.039). K562 stimulation resulted in higher CD107a expression on CD8 and NK cell subsets in the patients with NSCLC (p=0.018 and p=0.06).

Conclusion: Our findings showed that the CD8dim, suppressor $CD8^+$ $CD28^-$ T cell subgroup, as well as NKT and NK cells were increased but the percentages of B cells seemed to be decreased in patients with operable NSCLC. Increased $CD8^+$ $CD28^-$ T cells represent the main inhibitory phenotype in the immune system and their prevalence might be useful to assess immunotherapy outcomes in patients. The role of these cells in prognosis and the correlation of those rates with tumor stage are yet to be elucidated. Parameters of an immune score might be used for important prognostic factors for patients with newly-diagnosed NSCLC in the future.

Keywords: Non small cell lung cancer; cytotoxicity; T cells; cytotoxic T cells.

[P-0130]

[Tumor Immunotherapy]

Liposomal D-and K-type CpG ODN combination confers synergistic immune activation and act as an efficient tumor vaccine

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Delivering labile CpG ODNs within liposomes are advantageous. Liposomes protects the cargo from premature digestion, furthermore, it retains ODN activity/stability until it reaches relevant target cells. Although both are strictly dependent on TLR9, two structurally distinct classes of CpG ODNs are capable of activating human pDCs differentially. While K-type ODN trigger pDCs to differentiate, mature and secrete TNF-α, D-type ODN initiate IRF-dependent IFN-α production. Strikingly, when K- and D-type ODNs are co-incubated in their free forms, K-ODN masks the activity of D-ODN. However, to induce CpG ODN dependent-immunotherapy both K- and D-specific immune activations are indispensable. In this study, we hypothesized that encapsulating K-type and D-type CpG ODN in different liposomes could modify their subcellular distribution and lead to a synergistic immune activation rather than antagonistic obstruction. Data revealed that simultaneous stimulation with neutral D-ODN loaded liposomes along with K-ODN loaded within cationic liposomes improved rather than masking D-specific IFNα production from PBMCs. Of ~90% of all PBMCs reproduced this activation (n=10 donors). Intracellular cytokine staining and ELISPOT studies supported this heightened immune activation (TNF-α and IFN-α) of liposomal combinations of K- and D-type CpG ODNs. Similar findings were detected in mice experiments. Furthermore, the liposomal K- and D-ODN combinations were used in immunization studies against ovalbumin (OVA). Results implicated that when either neutral (ND) or anionic liposomal (AD) D-ODN is combined either with sterically stabilized cationic liposomal K (SSCL-K) or cationic-K-ODN (CK), anti OVA-specific IgG2a/IgG1 responses were over 1.0, thereby a pronounced Th1-biased anti-OVA immunity was established. To check whether this improved Th1-immunity is specific enough to control OVA-dependent B16 melanoma development in mice we next challenged immunized animals with OVA expressing B16 tumor cells. Only mice that were immunized with ND+SSCL-K vaccine combination, had the lowest tumor size (145.7±87.4) compared to naïve (1372.8±840.6) or unencapsulated D+K (410.9±287.1) immunized groups @ D=15 post-tumor challenge. This study demonstrated that superior features of D and K type CpG ODN could be combined if suitable liposomes were employed for loading, and extends the breadth of CpG ODN-dependent immunotherapy which otherwise is not possible. Overall, our data implicate that an effective preventive liposomal cancer vaccine against melanoma could be developed.

Keywords: Liposome; CpG ODN; tumor vaccination.

[P-0133]

[Tumor Immunology & Molecular Markers]

Investigation of miR-125 SNP (rs12976445) in prostate cancer patients

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Prostate cancer (Pca) is the most commonly diagnosed non-dermatologic malignancy and is the second leading cause of cancer death among men in the western countries, which are diagnosed at the age of 70 or even later. At this age, the incidence of prostate cancer is very high (3-70%). Quantitative and structural genetic alterations cause the development and progression of prostate cancer. A number of genes have been implicated in prostate cancer by genetic alterations and functional consequences of the genetic alterations.

MicroRNAs (miRNAs) are a class of small, non-coding, single-stranded RNAs that are approximately 22 nt in length, that negatively regulate of up to 30% of gene expression by mainly binding to 3'-Untranslated Region (3'-UTR) of target mRNAs at the post-transcriptional level. MicroRNAs (miRNAs) are play a role in many biological processes. miR-125 is a family of miRNAs that functions as either a tumor suppressor gene or oncogene by regulating different pathways in different types of tissues. The role of miR-125b in prostate cancer is controversial. Some studies have shown down-regulation of miR-125b in malignant prostate cancer. However, another study reported that androgen represses miR-125b expression, leading to up-regulation of a number of target genes. It was founded that miR-125b promotes carcinogenesis in myeloid leukemia, B-cell leukemia, non-small cell lung cancer, glioblastoma, clear-cell renal carcinoma, prostate cancer, pancreatic cancer and oligodendroglioma.

The aims of this study to identify the putative relationship between rs12976445 SNP in mir-125 and the prostate cancer progression, and evaluate the potential of diagnostic and prognostic markers for mirRNA. For this aim, we isolated DNA from blood taken from 30 patients with prostate cancer and 40 healthy subjects as a control group. The genotyping of mir-125 (rs12976445) SNP was analyzed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. In conclusion, increased ratio of C/C genotype in patient group was founded.

Keywords: miRNA-125; prostate cancer; polymorphism.

[P-0135]

[Tumor Immunotherapy]

Enhanced delivery and internalization of iron oxide nanoparticle-loaded exosomes

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Exosomes are a class of naturally occurring nanovesicles that are secreted virtually by all cell types. Recent studies revealed that due to their low toxicity/immunogenicity as well as their nanosize, exosomes are excellent vehicles for protein/peptide drug, gene or even oligonucleotide delivery. Targeted drug delivery proved to be a critical topic in achieving efficient cancer therapy. In recent decades, endogenous vehicles, such as protein and polysaccharide nanoparticles were explored extensively for drug delivery to obtain better therapeutic effects because of their low toxicity and biocompatibility. Here, we describe a mild method to externally load magnetic iron oxide nanoparticles (FeO NPs) within exosomes. The modified exosomes could be driven more effectively to target cells under magnetic field, thereby expand exosome directed therapy opportunities.

Exosomes were loaded with different doses of FeO NPs via rehydration-dehydration method. Internalization of FeO NP loaded and unloaded exosomes were conducted both under magnetic and no-magnetic field at various time intervals. In some experiments in addition to FeO NP loading D-type CpG ODN was also co-encapsulated within exosomes and internalization as well as stimulatory activities were analyzed either on RAW264.7 macrophages or on spleen cells.

Data revealed that exosome uptake by macrophages improved upto 35 fold in a dose dependent manner in magnetic field compared to exosomes devoid of FeO NP within 30 minutes of incubation. Moreover, co-encapsulation of D-type CpG ODN with FeO NP within exosomes significantly increased ODN internalization by 30 minutes of incubation especially at lower ODN dose in comparison to FeO NP free form of exosomes. Immunostimulatory activities of CpG ODN under magnetic field following co-encapsulation within exosomes along with FeO NP found to be significantly magnified in addition to rapid cytokine induction rate.

In conclusion, this study showed that FeO NPs increased internalization of exosomes, thereby facilitated rapid and intense delivery of its cargo to target cells under magnetic field. Loading FeO NPs within exosomes offers an effective theranostic approach for developing targeted exosomes that could sustain their translational impact.

Keywords: Exosome; nanoparticle; drug delivery; magnetic field; targeted therapy.

[P-0136]

[Inflammation and Diseases]

Investigation of SNP (rs10889677) located miRNA binding sites of the IL23R gene in patient with polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is a common endocrine system disorder among women of reproductive age. Women with PCOS may have enlarged ovaries that contain small collections of fluid called follicles-located in each ovary as seen during an ultrasound exam. Infrequent or prolonged menstrual periods excess hair growth, acne, and obesity can all occur in women with polycystic ovary syndrome. In adolescents, infrequent or absent menstruation. may raise suspicion for the condition. The exact cause of polycystic ovary syndrome is unknown. Early diagnosis and treatment along with weight loss may reduce the risk of long-term complications, such as type 2 diabetes and heart disease. The cause of polycystic ovary syndrome (PCOS) is not fully understood, but genetics may be a factor.

MicroRNAs (miRNAs) are small, non-coding RNAs that are able to regulate gene expression at the post-transcriptional level. Altered miRNA levels have been associated with diabetes, insulin resistance, inflammation and various cancers. Studies have shown that circulating miRNAs are present in whole blood, serum, plasma and the follicular fluid of PCOS patients and that they might serve as potential biomarkers and a new approach for the diagnosis of PCOS. Research into the etiology has recently focused on the role of the immunity and inflammation. Interleukin-23 (IL23) and their receptors (IL23R) guide T cells towards the phenotypes. IL23R single nucleotide polymorphisms (SNPs) have been shown to be associated with digestive system cancers. A variant of the IL-23R gene associated with inflammatory bowel disease induces loss of microRNA regulation and enhanced protein production. In our study we investigated the putative relationship between SNP (rs10889677) in the 3'-UTR region of the IL23R and pathogenesis of PCOS. This SNP miR- Let-7e (let-7f) binding sites of IL23R gene.

For this aim, we will collect the blood from 79 subjects, and isolate DNA. After PCR amplification, the genotyping was done by Polymerase Chain Reaction-Restricted Fragment Lenght Polymorphism (PCR-RFLP) method. Finally the results was analyzed by statistically.

Keywords: PCOS; miR- Let-7e; polymorphism.

[P-0137]

[Tumor Immunology & Molecular Markers]

Effect of rapamycin on mRNA expression of IL-8 and CXCR1 in HER2+ breast cancer cells

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Aim: Up to 25% of breast cancers overexpress human HER2 receptor tyrosine kinase, which leads to a higher rate of recurrence. HER2-HER3 heterodimer is the most potent oncogenic dimer in breast cancer. In addition, it has been shown that co-expression of HER2 and HER3 receptor tyrosine kinases enhances invasion of breast cancer cells via stimulation of interleukin IL-8 secretion. IL-8 signals via two cell surface receptors, CXCR1 and CXCR2. In breast cancer, PI3-K/Akt, HER2/3 and IL-8/CXCR1 signaling pathways seem to be closely linked. Previously, our laboratory demonstrated a transient decrease in mRNA expression of IL-8 and CXCR1 after Akt inhibition via AKT-i (a pan-Akt inhibitor). The aim of this study is to determine the effects of rapamycin (an mTOR inhibitor) on IL-8/CXCR1 at transcriptional level.

Material and Methods: SKBR-3 breast cancer cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS (fetal bovine serum) and 1% penicillin/streptomycin. Cells were treated with 50 nM rapamycin for 8 and 24 hours; mRNA expressions were examined using qPCR. Student's t test was performed, $p<0.05$ was assumed statistically significant.

Results: Rapamycin treatment of SKBR-3 cells for 8 and 24 hours, did not change IL-8 mRNA expression significantly. Besides, exposure of SKBR-3 cells to rapamycin for 8 hours increased HER2 and HER3 mRNA expressions significantly. Incubation of SKBR-3 cells with rapamycin for 24 hours increased CXCR1, HER2 and HER3 mRNA expressions significantly.

Conclusions:

1) IL-8 mRNA expression is not affected from HER3 up-regulation via rapamycin in SKBR-3 cells despite the fact that this translational up-regulation and activation of HER3 is known to be present at least for 72 hours.

2) Rapamycin effect on mRNA expressions of HER2 and HER3 is compatible with translational up-regulation of HER3 in these cells.

3) The increase in CXCR1 mRNA levels after 24 hours of mTOR inhibition may be due to possibly increased mRNA stability which needs further investigation.

4) Rapamycin effect should be further analyzed at translational level to evaluate the functional consequence on IL8/CXCR1 signaling in breast cancer cells.

Keywords: HER2; HER3; mTOR; IL-8; CXCR1; rapamycin.

[P-0142]

[Inflammation and Diseases]

Detection of anti-Brucella antibodies by an antigen obtained from Rhizobium tropici

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Laboratory diagnosis of brucellosis is made by serological, molecular and cultural methods. *Brucella spp., Agrobacterium, Ochrobactrum* and Rhizobium all belong to the alpha-2 subgroup of Proteobacteria as revealed by studies on 16S rRNA sequence analysis. *Ochrobactrum anthropi* has been used to diagnose of brucellosis.

In this study, whole cell extract from *Rhizobium tropici (R. tropici)* was examined for detecting antibodies to *B. abortus* and *B. melitensis* in cattle and sheep sera, respectively. Genetic similarity between the bacteria *R. tropici, B. abortus and B. melitensis* was also investigated by RAPD-PCR method. Using the sera from cattle, sensitivity and specificity were calculated as being 81.1% and 22.6%, respectively when the Brucella serum agglutination test considered as Gold Standard. Rhizobium slide agglutination test (RSAT)'s sensitivity for sheep sere (80.1%) was found being close to that of cattle sera (81.1%) while specificity of RSAT for sheep sera (59.5%) was quite higher than the figure for the cattle sera (22.6%).

In conclusion, use of whole cell antigen from *R. tropici* is not practical in sero diagnosing brucellosis in cattle and sheep. It is also considered that some antibodies to *R. tropici* are also mounted in the hosts contributing misunderstanding of the positivitiness in the serology.

Keywords: Brucellosis; rhizobium tropici; serology; antibody.

[P-0143]

[Immunity to Infection: New Trends and Developments]

Prevalence of clostridium difficile and effect of the antibiotic therapy in hospitalized children

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Aim: To determine the prevalence of Clostridium difficile in hospitalized children and investigate the effect of the antibiotic therapy.

Material-Methods: Total of 106 patients were enrolled in this study, which initially hospitalized with different diagnosis. Stool samples were tested for Clostridium difficile A/B toxin from every patient during the hospitalization. Patients with initial negative test results and those received antibiotic therapy during admission were retested immediately before discharge.

Results: Clostridium difficile A/B toxin was found to be positive in 24.5% of the total study population. The prevalence of Clostridium difficile positive group (31.9%) was significantly higher ($p=0.01$) in patients that had an antibiotic use prior to hospitalization compared to the group without

prior antibiotic use (8.8%). 88.4% of the patients who tested positive for Clostridium difficile toxin during the hospitalization had a prior history of antibiotic usage, was significantly higher (p=0.01) than the patients without prior antibiotic use (61.2%). Of the patients who were initially tested negative and received antibiotics during hospitalization, 6.1 % tested to be positive immediately before discharge. Prevalence of the positive test results was higher (48.1%) in patients admitted to the Hematologic-Oncology service.

Conclusions: Clostridium difficile prevalence is higher in children. It has to be considered as part of the differential diagnosis in patients presenting with fever, diarrhea and abdominal pain. Excessive use of antibiotics must be avoided in general and child population. Patients with Clostridium difficile infection must be isolated from the rest of the service and contact precautions should be implemented.

Keywords: Clostridium difficile; toxin; children.

[P-0144]

[Tumor Immunology & Molecular Markers]

A possible role for B7 family of costimulatory molecules in the immunogenicity of small cell lung cancer cells

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Introduction and Aim: Small cell lung cancer (SCLC) which comprises 13% of all lung cancer cases is associated with aggressive tumor growth together with a tendency to early dissemination and distant metastasis. Yet, current knowledge concerning the interaction between SCLC cells and immune system components are limited. Especially, certain types of cancers are known to express B7 co-stimulatory molecule family members that are able to directly modulate T-cell responses. Co-inhibitory members of B7 molecules (B7-H1, B7-H3, B7-DC, B7-H4, B7-H5) are implicated in tumor escape whereas co-stimulatory members (B7-1, B7-2, B7-H2, B7-H6) were associated with cancer immunogenicity and adaptive resistance mechanism. Thus, the aim of this project is to determine the effect of SCLC cells on T cell responses and to associate them with the presence of B7 family members. Expression of these molecules under proinflammatory conditions were also determined.

Methods: Three SCLC cell lines (SCLC-21H, NCI-H69, NCI-H82) were cultured under standard conditions or in the presence of proinflammatory cytokines IFN-gamma, TNF-alpha, IL-1 beta or IL-6. Expression of B7-1, B7-2, B7-H1, B7-H2, B7-H3, B7-H4, B7-H5, B7-H6 and B7-DC molecules were assessed at mRNA level RT-PCR and at surface protein level with flow cytometry. Proliferation (eFluor-647 flow cytometric assay) capacity of PBMCs, monocyte-depleted PBMCs or purified CD4+ or CD8+ T-cells were determined upon co-culturing with different ratios of SCLC cells in the presence of anti-CD3 mAb.

Results: SCLC cell lines highly expressed co-stimulatory B7-H2 and B7-H6 molecules together with co-inhibitory B7-H3 and B7-H5. B7-H3 was prominently detected on all SCLC cells. Additionally, B7-1, B7-2 or B7-H1 expression was scarce and only very small subpopulation (<8%) was positively stained for these markers. In contrast to the literature on several other cancer types, B7 molecules' expression was not essentially modulated under the influence of pro-inflammatory cytokines. Intriguingly, co-culture of SCLC cells with PBMCs (especially when monocytes were not depleted) augmented T cell proliferation suggesting an immunogenic action by these cancer cells. However, CD4+ or CD8+ T-cells cocultured with SCLC cells did not lead to T cell proliferation suggesting an indirect interaction between SCLC cells and T cells through monocytes.

Conclusion: Understanding the mechanisms underlying the immunogenicity of SCLC cells and how they manage to escape immune destruction is of interest. Our current findings indicate a possible role for B7 family members in this process.

Keywords: SCLC; T-cell activation; co-stimulation; B7 family.

[P-0145]

[Immunodeficiencies: Emerging Molecular & Cellular Therapies. Remembering Prof. Isıl BARLAN]

Detection of genetic variations in severe combined immunodeficiency (SCID) patients by targeted amplicon sequencing

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Primary immunodeficiencies (PIDs) are inherited disorders in which one of more components of the immune system are missing or do not work properly. Severe combined immunodeficiency (SCID) is one of the most severe forms of PID, which is characterized by the dysfunction and absence of T lymphocytes. Delay and improper diagnosis of SCID can resulted in life-threatening situation and curative treatments can only be achieved by bone marrow transplantation and gene therapy. Recently next generation sequencing (NGS) Technologies have been proven to be beneficial in medical diagnostics and NGS has became to be a rapid, high-throughput and low-cost sequencing technology, which has been used to identify novel mutations in several types of inherited diseases including SCID. In this study, a targeted next generation sequencing panel was developed for diagnosis of SCID patients using Smartchip-TE technology (WaferGen Bio-Systems Inc.) and Illumina Miseq system (Illumina Inc.). Eighteen (total of 376 amplicons) candidate genes were screened in 9 patients and the variants are determined by bioinformatics analysis. In our initial analysis results, we have found pathogenic variants in 3 out of 9 patients. Compound heterozygosity in RAG1 was detected in a 10-year-old female T- B- NK+ (leaky) SCID and 3-year-old male T- B- NK+ SCID patient. In the 10-year-old SCID

patient, the variants rs121918568 and rs104894289 were observed, while in the 3-year-old SCID patient, the pathogenic variants of rs372110434 and rs19947685 were detected. We also observed in an one year old T- B- NK+ SCID male patient with a novel homozygous stop codon variant in DCLRE1C named NM_001033857:c.200 T>G. This novel pathogenic variant was determined by in silico bioinformatics prediction tools. Currently we are still analyzing the NGS data to find the potential pathogenic variants in the other patients. Pathogenic variants are being confirmed by sanger sequencing.

Keywords: Primary immunodeficiency; SCID; next generation sequencing.

[P-0146]

[Tumor Immunology & Molecular Markers]

Semophorin 5A as a biomarker for colorectal cancer prognosis

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Introduction: Cellular stress occurs with uncontrolled proliferation and inhibition of controlled cell death (apoptosis), resulting in uncontrolled proliferation of colon epithelial cells. Genetic predisposition with well-described mutations play a major role for some colon cancers presenting as developing polyps from the epithelial lumen of the colon. To follow clinically diagnosed colorectal cancer, we propose Semaphorin 5A (SEMA5A) as a prognostic biomarker. Our candidate gene encodes a membrane protein in vertebrates, located on chromosome 5 with 21 exons. Its 1074 amino acid protein product binds to Plexin-3 receptors and is described as pro-angiogenic by increasing endothelial cell proliferation and decreasing epithelial apoptosis.

Methods: We first examined the gene expression levels of SEMA5A in age and sex-matched normal colon and colon cancer patients with real time-quantitative PCR with Taqman probes, using 18SrRNA as reference gene for gene expression calculations. We also examined the PCR products in agarose gel electrophoresis.

Results: A statistically significant increase in SEMA5A expression levels was observed in half of the twenty colorectal cancer tumor patients as preliminary results.

Conclusion: We are currently investigating SEMA5A protein levels in patient serum samples and comparing them with normal donors, before correlating our results with patient cancer-specific survival.

Keywords: Biomarker; colorectal cancer; SEMA5A.

[P-0147]

[Immunodeficiencies: Emerging Molecular & Cellular Therapies. Remembering Prof. Isıl BARLAN]

Intracellular staining of DOCK8, LRBA, SAP and XIAP proteins by flow cytometry; Marmara experience

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Aim: Primary immunodeficiency diseases are serious public health problems in Turkey. Accurate and early diagnosis is critical for these patients for appropriate therapeutic options. In this study, optimization and standardization of intracellular DOCK8, LRBA, SAP and XIAP protein expressions in heathy controls with a single flow cytometer protocol were provided. In addition, the protocol was also used in the diagnosis of clinically relevant patients.

Methods: Heparinized blood samples from patients and healthy controls were drawn. Peripheral blood mononuclear cells (PBMC) were immediately isolated. Then, DOCK8, LRBA, SAP and XIAP protein expressions were determined in PBMCs by flow cytometer. The data were analyzed as mean fluorescein intensity difference (ΔMFI) according to the isotype.

Results: ΔMFI values obtained by DOCK8 antibody staining were determined as 21.3±4 in CD3+T cells and 25±3.3 in CD20+T cells in healthy controls. Patients with DOCK8 deficiency showed values either very low or completely absent. ΔMFI values obtained by LRBA antibody staining were observed as 36±7.7 in healthy controls, while those values in the LRBA deficiency patients were identified at very low levels of 5.9±1.8. These values obtained by SAP and XIAP antibody staining were determined as 30.2±3 in CD8+T cells for SAP, 13.9±3.2 in CD3+T and 14.6±3.5 in CD20+B cells for XIAP.

Conclusion: Due to its rapid and reliable results in clinically relevant cases for DOCK8, LRBA, SAP and XIAP, analysis of intracellular protein expression by flow cytometry can be used as a diagnostic procedure. Patients with remarkably decreased protein expressions or ΔMFI values compared to simultaneous healthy controls and isotype controls should be confirmed with gene sequencing.

Keywords: Flow cytometer; intracellular protein expression; mean fluorescence intensity; DOCK8 deficiency; LRBA deficiency.

[P-0148]

[Immunodeficiencies: Emerging Molecular & Cellular Therapies. Remembering Prof. Isıl BARLAN]

Basophil activation test is reliable for inhalant allergens in pediatric patients

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Background: Flow cytometric quantification of *in vitro* basophil activation can be quite performant and reliable tool to measure IgE-dependent allergen-specific responses in allergic patients. The aim of this study was to determine sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) of BAT in our patients sensitized to house dust mite (HDM) and grass pollen with allergic rhinitis and/or asthma.

Methods: Forty-seven children (35 M/ 12 F, 11±3 years) sensitized to house dust mite and grass pollen with a clinical history of allergic rhinitis and/or asthma were enrolled. To confirm sensitization, a positive skin prick test (SPT) or elevatated specific IgE to Dermatophagoides farina (DF), Dermatophagoides pteronyssinus (DP) and grass pollen were used. The non-allergic healthy control group (n=18) had no history of allergy with negative skin prick tests to the common allergens tested. The BASOTEST kit (Glycotope Biotechnology GmbH, Heidelberg, Germany) was used to carry out BAT according to the manufacturer's instruction. Basophil activation was determined by flow cytometry upon double staining with anti-IgE/anti-CD63 mAb. The upregulation of the activation marker CD63 was calculated by the percentage of the CD63-positive cells compared with the total identified basophilic cells.

Results: Receiver operating characteristics (ROC) curve analyses yielded a cut-off level for positive response as 15% for BAT with HDM and grass pollen when patients and healthy controls were all evaluated. For HDM, the sensitivity, specificity, PPV, and NPV of the basophil activation were 90%, 73%, 0.70 and 0.91, respectively. The analysis of concordance of being either allergic or healthy in comparison to BAT results for HDM revealed a substantial concordance (κ index= 0.61, $p<0.001$). For grass pollen, BAT attained a sensitivity, specificity, PPV, and NPV of 96%, 93%, 0.98 and 0.88, respectively. The analysis of concordance of being either allergic or healthy in comparison to BAT results for grass pollen revealed an almost perfect concordance (κ index= 0.87, $p<0.001$).

Conclusion: Our results confirmed BAT as reliable and easy method to detect basophil activation in response to grass pollen and HDM in pediatric inhalant allergic patients. The obtained specificity, sensitivity, PPV and NPV values for these allergens were also found to be comparable with the levels in the literature.

Keywords: Basophil activation test; CD63; house dust mite; grass pollen; sensitivity; specificity.

[P-0149]

[Transplantation Immunology]

Investigating the effects of AT1R on organ rejection in patients with kidney transplantation

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Objectives: Kidney transplantation is the most effective treatment modality in end-stage renal failure. The risk of organ rejection is significantly decreased by transplanting from HLA-matched donor as well as detecting antibodies against HLA structure (panel reactive antibody; PRA). The effects of the presence of non-HLA Ab on kidney transplantation still keep indeterminateness.

The angiotensin-II type 1 receptor (AT1R) is main receptor for angiotensin-II in glomeruli and responsible for the regulation of arterial blood pressure and salt balance. Overactivation of angiotensin II-AT1R system leads to hypertension and cardiac and renal complications due to vascular remodeling. In renal transplantation patients with severe vascular rejection and malign hypertension and lack of anti-HLA antibody, agonistic AT1R-Ab (AT1R antibody) has been detected. The binding of AT1R-Ab to AT1R is considered as a critical stage for post-transplant renal damage. The aim of this study is to determine the AT1R-Ab levels, to evaluate the relationship between AT1R-Ab levels and their effects on appearing rejection.

Methods: The study has been performed with 150 subjects: 100 patients (including 30 patients with rejection and 70 with normal course after transplantation) and 50 healthy controls. Rejection group is composed of 9 acute humoral, 14 acute cellular, 4 mix type, 2 chronic and 1 infectious related rejection. Patient group had been selected among kidney transplanted patients in Başkent University Adana Research and Medical Center between 2010 and 2015. Patient group includes 69 adult (16 female, 53 male) and 31 pediatric patients (12 female, 19 male). As healthy controls, 50 healthy volunteers (15 female, 35 male) were included in the study.

For both groups collected sera were quantified by using AT1R assay kits (One Lambda, Calif, United States) with the ELISA method (cut off value: 17 U/mL was selected with reference to the manufacturing firms instructions). For all transplantation patients, in pretransplant and posttransplant stage, ATR1-Ab measurements were evaluated. In patient with rejection in rejection period additional ATR1-Ab levels were measured too. Statistical analysis was performed using the statistical package SPSS software (Version 17.0, SPSS Inc., Chicago, IL, USA).

Results and Conclusions: AT1R-Ab levels of pre transplantation stage, of post transplantation stage, of rejections stage and of healthy controls are given in the table below. Pretransplant AT1R-Ab positivity is a risk factor for acute rejection and associated with antibody mediated rejection.

Keywords: AT1R; transplantation; rejection; non HLA antibodies.

Table 1. AT1R-Ab levels

AT1R Ab levels (U/ml) in subjects	Pretransplantation	Posttransplantation	Rejection	Healthy controls	P value
Adult Mean±SD	n:42 3,23±1,1	n:42 4,51±3,2	n:27 6,15±3,4	n:50 3,14±0,5	0,0001
Pediatric Mean±SD	n:32 6,25±3,7	n:27 6,68±2,9	n:4 7,12±6,17	-	0,424

[P-0150]

[Novel Discoveries in the Innate Immune System]

pH sensitive nanoliposomes co-encapsulated with cyclic di-nucleotides and CpG ODN enhances Th1-biased immunity

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STING ligands (cyclic dinucleotides, CDNs) induces IRF3 and NFKβ pathways activation via binding to the adaptor protein STING on ER and leads to the secretion of type I IFNs and modulates T cells activation. Recent studies suggested that CDNs are potent immunoadjuvants or immunotherapeutic agents. However, their *in vivo* performance is limited due their cyclic structures, which prevent their uptake by immune cell. Recently our group demonstrated that there is a synergy of the generated immune response when combinations of CDNs and TLR9 ligands (CpG-ODN) were used. In this study, we co-encapsulated STING and TLR9 ligands within pH-sensitive liposomes and aimed to enhance this synergy further. We postulated that liposomes will increase the cellular uptake and subsequently deliver CDN into cytosol while retaining CpG-ODN at the site of TLR9 in late endosome. Splenocytes were stimulated with free or liposomal CpG-ODN (K3) and/or CDNs (c-di-GMP or 2'3' cGAMP) for 24h. Cytokine production (IL6, IL12, IFNγ and IFNα/β) from splenocytes were detected by ELISA and IFNα/β by reporter cell line (B16- IFNα/β).

Our findings revealed that liposome encapsulation boosted IL6, IL12, IFNγ as well as IFNα/β levels upon CDNs+ODNs stimulation, compared to their free counterparts or their free combinations. The synergy was observed at low doses where free ligands could not induce any stimulatory effect.

This study demonstrated that while free ligands (CDN+ODN) at low doses had no immunostimulatory effects, their liposomal formulations induced robust type I and II IFNs by enhancing their sub-cellular distributions and initiated more stronger STING and TLR9-triggered immune response. These formulations could be considered as anticancer or antiviral agents. Studies to prove the *in vivo* benefit of liposome co-encapsulation is underway.

Keywords: CpG ODN; STING; CDNs; liposome; adjuvant; vaccine.

[P-0152]

[Inflammation and Diseases]

The usefulness of *in vitro* tests for the diagnosis of severe drug hypersensitivity reactions: a case report with ulcerative colitis

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A 22-year-old young man with a two-year history of ulcerative colitis (UC) was evaluated after developing fever, malaise and severe pericarditis following one-day treatment with 5-aminosalycylic acid, sulfametoxazole, iron and aspirin. After the exclusion of other infective or UC related complications, drug induced pericarditis was suspected. The general health status of the patient required an *in vitro* approach for the identification of the culprit drug.

Basophil activation test (BAT) and lymphocyte transformation test (LTT) were carried on the patient and on two age and gender matched controls (one healthy control and one patient with UC) and comparisons between them are shown. LTT was analyzed in order to determine drug-specific CD4+ T cells. Unstimulated and stimulated with suspected drugs, carboxyfluorescein succinimidyl ester (CFSE)-labeled PBMCs were cultured for five days and drug specific CD4+ T cells were determined by flow cytometry. The BAT (Flow CAST®) was carried out according to manufacturers instructions. Samples were prepared with suspected drugs for case and controls, in validated concentrations and incubated at 37°C for 15 minutes. The basophils were labeled staining reagent containing a mixture of anti human-CD63, -203c and -CCR3 monoclonal antibodies and basophils were analyzed by flow cytometry. Iron-specific CD4+ T cell proliferation greater than 1.5 times proliferation of unstimulated cells was accepted as positive. At the patient's analysis, T cell proliferation was increased by the addition of iron with folds 1,7. BAT remained negative for the case and the controls for suspected drugs. LTT with sulfametoxazole, salicylic acid, aspirin and iron were found negative. *In vitro* tests for uncommon life threatening drug hypersensitivity reactions have most importance for the diagnosis and need further improvement.

Keywords: Drug hypersensitivity; lymphocyte transformation test; basophil activation test.

[P-0157]

[Transplantation Immunology]

Evaluation of the cytokine gene polymorphisms on rejection in kidney transplantation

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Objectives: Cytokines are essential mediators for immune system responses. Secreted cytokines bind to their corresponding receptors in target cells and regulate cellular functions. Cytokine gene polymorphisms can modulate the cytokine production rates, their affinity to the corresponding receptors and their activities. Cytokine gene polymorphisms have been investigated in patients with kidney transplantation. Many studies report a relationship between

these polymorphisms and renal damage or prevention of graft loss. Cytokines, which play important roles in allograft rejection, show variable production among individuals. These variations may be related to genetic polymorphisms within the regulatory regions of the cytokine genes.

The aim of this study is to determine the gene polymorphisms which are consistent with low, medium and high secretion of 5 cytokines representing pro-inflammatory and anti-inflammatory groups, to their effects on appearing rejection. We investigated the association between the role tumor necrosis factor alpha (TNF-α), transforming growth factor-beta (TGF-β), interferon gamma (IFN-γ), interleukin (IL)-10 and IL-6 gene polymorphisms and graft rejection among kidney transplant recipients.

Methods: The study has been performed with 150 subjects: 100 patients (including 30 patients with rejection and 70 with normal course after transplantation) and 50 healthy controls. Patient group had been selected among kidney transplanted patients in Başkent University Adana Research and Medical Center between 2010 and 2016. For this purpose, DNA was isolated from blood samples with EDTA from both patients and healthy control groups. To determine the targeted polymorphic regions of DNA which is replicated by sequence-specific primers (SSP)-PCR method, DNA bands determined by gel electrophoresis system was analyzed. Genotyping of the following positions: TNF-α (-308G/A), TGF-β1 (codon 10T/C, codon 25C/G), IL-10 (-1082G/A, -819C/T, -592C/A), IL-6 (-174C/G), and IFN-γ (+874T/A) were performed.

Statistical analysis was performed using the statistical package SPSS software (Version 17.0, SPSS Inc., Chicago, IL, USA). Pre-post measures data were analysing Repeated Measure Analyses. Values of $p < 0.05$ were considered statistically.

Results and Conclusions: The frequencies of IL-10 GCC/GCC (High), TGF-β1 T/T G/G (High) and TGF-β1 T/C G/G (High) polymorphisim were significantly higher in non rejection group patients when compared with rejection group patients. IL-6 C/C (Low) polymorphisim was significantly higher (p=0.018) in rejection group patients when compared with non rejection group patients. These results suggest that IL-10, TGF-beta1 and IL-6 gene polymorphisms may affect immune response and the outcome of the renal transplant patients.

Keywords: Kidney transplantation; cytokine gen polymorphism; rejection.

[P-0158]

[Tumor Immunology & Molecular Markers]

Proliferative response of CD4+ peripheral Helper T-cells to fibroblasts obtained from lesional and normal skin of mycosis fungoides patients

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Introduction: Mycosis fungoides (MF) is the most common primary cutaneous T-cell lymphoma. It usually consists of atypical CD4+CD45RO+ T-lymphocytes. The interaction between malignant cells and their microenvironment affects tumoral expansion and progression. Cancer associated fibroblasts (CAFs), a component of tumor microenvironment, are activated fibroblasts supporting tumor progression. In this study, we aimed to investigate the effects of cancer associated fibroblasts obtained from lesional skin of mycosis fungoides patients on the proliferation of CD4+ peripheral helper T-cells and to compare this effect with that of normal dermal fibroblasts.

Materials and Methods: Four patients with a diagnosis of MF were included in the study. After obtaining written consent, two skin biopsies were taken from lesional and normal skin of the patients. Tissue samples were divided into 2-3 mm pieces and one piece was placed in each well of 24-well tissue culture plates. High glucose DME medium containing 10% FBS, 1% L-glutamine and 1% penicillin/streptomycin was used. When the confluence of the fibroblasts reached 80-90%, the cells were trypsinized and passages to 25 cm^2 tissue culture flasks. Only fibroblasts at first passage were used for coculture experiments to prevent loss of CAF phenotype. The fibroblasts were characterized through immunocytochemistry. Peripheral T-helper lymphocytes were obtained from peripheral blood of patients using magnetic microbeads. They were then stained with carboxyfluorescein succinimidyl ester, activated with anti-CD3/CD28 magnetic microbeads, and were cocultured with cancer associated or normal dermal fibroblasts in 96-well tissue culture plates. Lymphocytes with and without addition of activator microbeads were cultured alone to be used as positive and negative controls, respectively. Proliferation rates of lymphocytes were assessed through flow cytometry.

RESULTS: Cancer associated stained positive with anti-α-SMA antibody whereas normal fibroblasts were negative. Fibroblasts suppressed proliferation rates of CD4+ peripheral T-helper cells. Normal dermal fibroblasts were found to suppress proliferation more than cancer associated fibroblasts.

Keywords: Mycosis fungoides; fibroblasts; tumor microenvironment.

[P-0160]

[Tumor Immunology & Molecular Markers]

Myeloid cells under the influence of basal-like breast cancer: a possible link between, IL-1β, fibronectin and STAT3

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Introduction and Aim: Myeloid cells infiltrating the tumor tissue undergo tumor-mediated immune regulation which supports cancer progression. Interactions with the extracellular matrix components, especially fibronectin, and activation of STAT3 transcription factor can modulate the character of myeloid cells. Pro-inflammatory cytokines influence extracellular matrix composition in tumor microenvironment. Here, effect of basal-like breast cancer on myeloid cell character and regulation of IL-1β mediated fibronectin production of cancer cells was investigated.

Methods: Myeloid cell lines (U937 and THP-1) were cultured in conditioned-media obtained from luminal or basal-like type of breast cancer cells (MCF-7, BT-474, SKBR-3, MDA-MB-468, MDA-MB-231 and HCC38). In the presence or absence of a STAT3 inhibitor (Stattic), STAT3 activation were evaluated in myeloid cells by Western-Blot. The changes in fibronectin receptor (integrins α4, α5, αX, αM, β1, β3, β7) expression were determined by real-time RT-PCR. Breast cancer cell lines were stimulated with IL-1β and fibronectin secretion was analyzed by ELISA.

Results: High levels of fibronectin expression was determined in basal-like breast cancer cells (MDA-MB-231 and HCC38). Fibronectin secretion was enhanced with IL-1β in breast cancer cells. The integrin subunits of fibronectin receptors tend to increase in myeloid cells incubated with basal-like breast cancer supernatants. The factors secreted form MDA-MB-468, MDA-MB-231 and HCC38 basal-like cells augmented STAT3 activation (pSTAT3 (Tyr705)) in myeloid cells. On the other hand, luminal breast cancer cells (MCF-7, BT474, T47D, ZR75-1 and SKBR-3) did not induce pSTAT3. Phosphorylation of this transcription factor was efficiently inhibited with Stattic in myeloid cells treated with conditioned media from basal-like breast cancer cells.

Conclusion: Basal-like breast cancer cells can effectively stimulate STAT3 pathway which leads to a regulatory phenotype in myeloid cells. Inhibition of pSTAT3 activities can be a strategy to interfere with tumor-associated features of myeloid cells in basal-like breast cancer. Fibronectin production in basal-like breast cancer cells is regulated by IL-1β. IL-1β might serve as a factor produced by myeloid cells that maintains chronic inflammatory state in the tumor microenvironment.

This study is supported by TUBITAK (Project no: 113Z923).

Keywords: Myeloid cell; basal-like breast cancer; STAT3; fibronectin; IL-1β.

[P-0164]

[Tumor Immunotherapy]

The effect of ionizing radiation and glucocorticoid induced tumor necrosis factor ligand (GITRL) expression in basal-like breast cancer cells

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Introduction and Aim: Basal-like breast cancers are acknowledged to be enriched in cancer initiating cells with enhanced proliferation and differentiation potential into tumorigenic and non-tumorigenic cells and are resistant both to chemo- and radiotherapy. Double-strand breaks (DSBs) inflicted by ionizing radiation induce cancer initiating cells go into cell cycle arrest and DNA repair. As far as DSBs are concerned, most significant primary transducer is Ataxia Telangiectasia Mutated (ATM) protein which is a central activator of DNA damage response pathway. Glucocorticoid Induced Tumor Necrosis Factor Ligand (GITRL) is also upregulated under stress conditions and have modulatory effects on immune responses, especially on regulatory T cells (Treg) cells. This study aims to determine a possible link between ionizing radiation, ATM phosphorylation and GITR/GITRL pathway in basal-like breast cancer.

Methods: GITR and GITRL expression was determined in breast cancer cell lines of luminal type (MCF-7, BT-474, SKBR-3) and of basal-like type (MDA-MB-468, MDA-MB-231, HCC-38). Basal-like breast cancer cells were exposed to 5 Gy and 10 Gy ionizing radiation and the induction of ATM phosphorylation was determined by Western-Blot analysis.

Results: Without ionizing radiation, HCC38 cell line shows high expression of GITR and subtle expression of GITRL, when compared with other breast cancer cell lines. In the basal breast cancer cell lines (MDA-MB-468, MDA-MB-231, HCC-38), ATM expression and its phosphorylation state is altered with 5Gy and 10Gy ionizing radiation. Accordingly, these cells demonstrated high expression of phospho-ATM following treatment with after ionizing radiation.

Conclusion: These preliminary results indicate that inclusion of ATM promoter as a control element on recombinant DNA constructs can form a switch mechanism for ionizing radiation. Introduction of GITRL gene under the switch control of ionizing radiation may have implications in breast cancer gene therapy.

Keywords: GITRL; ionizing radiation; ATM; basal-like breast cancer cells.

[P-0165]

[Immunity to Infection: New Trends and Developments]

Assessment of knowledge about the BARC Center and satisfaction with the educational services available

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The aim if this proposal is to determine knowledge in the general population about arthritis and immunology disorders; to ascertain if this has improved in the decade since our Needs Assessment; and the satisfaction with the educational material now available to support communities and arthritis sufferers.

Background: BARC (Birmingham Arthritis Resource Centre) was set up to provide education and support to people with arthritis and their carers, based on a formal Needs Assessment. BARC aims to promote self-coping-to help people to deal with the physical and social disabilities caused by their disease.

Photo of Poster
Synovial fluid accumulation in his right hand
Haber den Node the second finger of the right hand
Barchi node in the third finger of the right hand
Dactylitis finger in the second and fourth of the left leg
Dactylitis finger in the second and fourth of the left leg

The all Photo Case presentation for Abstract poster.

Hypothesis: We propose that the population in general tend to downplay the importance of their musculus-skeletal problems. This is reinforced by the poor publicity that arthritic diseases get compared to some others.

Methods: The first aspect will be carried out by collecting data about knowledge of BARC and satisfaction with current educational support using standardized questionnaires.

Broader aspects of Fellowship: The advantages of taking on this project would be to widen your experience into qualitative research and introduce you to a new but important area of Rheumatology, patient education.

Keywords: Immunodeficiency; osteoarthritis; autoimmunity; inflammatory arthritis.

[P-0166]

[Cellular and Molecular Targets in Rheumatoid Arthritis and Autoimmune/Autoinflammatory Diseases]

Comparison of active *Helicobacter pylori* infection rate and CagA virulence marker positivity in patients with various autoimmune diseases; first results

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The etiology of most autoimmune diseases remains elusive. Multiple publications have reported a role for *Helicobacter pylori* infection in causing a variety of extra-intestinal manifestations such as systemic lupus erythematosus and rheumatoid arthritis. There are many proposed pathways by which *H. pylori* may cause loss of self-tolerance. Cytotoxin-associated gene A (cagA) positive *H. pylori* strains cause more severe inflammation. This study was conducted to determine relationship with active *H. pylori* infection rate and CagA-IgG positivity among autoimmune diseases.

A total of 86 adult patients included the study with a diagnosis of various autoimmune diseases (35 Rheumatoid arthritis, Scleroderma 14, Systemic lupus erythematosus 10, Ankylosing spondylitis 9 and others 18) as patient group prospectively. A questionnaire (age, sex, occupation, living area, body mass index, blood group, systemic illnesses, gastrointestinal disease history, *H. pylori* eradication story, allergic diseases, education level, antidepressant usage, the number of households, CBC counts etc.) was applied. Age and sex-matched healthy subjects have included in the study as control group (not completed). Serum and fecal samples were collected from both study groups and stored at -80 °C. *H. pylori* stool antigens were detected by an immuno-chromatic commercial kit (HpSA, TOYO) according to manufacturer's directions and quantity of *H. pylori* IgG and Anti-CagA IgG was determined by EIA (Dia.Pro). Unfortunately, the analyses of control group are incomplete yet.

Table 1. Patients with autoimmune diseases and test results

	N (%)	Mean age (±SD)	HP-Stool Ag positivity (Active infection) N (%)	HP IgG Positivity N (%)	HP Anti-CagA IgG Positivity N (%)
Patients with autoimmune diseases		51.4(±15.3)			
Rheumatoid Arthritis	35 (40.7)		10 (13.0)	26 (31.0)	14(21.5)
Ankylosing spondylitis	9 (10.5)		3 (3.9)	5 (6.0)	1(1.5)
SLE	10 (11.6)		3 (3.9)	8 (9.5)	1(1.5)
Scleroderma	14 (16.3)		4 (5.2)	12 (14.3)	5(7.7)
Others (Sjogren's, Wegener, Takayasu polymyositis/dermatomyositis, Behçet's Disease)	18 (20.9)		7 (9.1)	18 (21.4)	10(15.4)
Total	86 (100)		27 (35.1)**	69 (82.1)	31 (47.7)
Control (Healthy individuals)	*	*	*	*	*
Microbiology laboratory data (gastroenterology samples, same method) 2015	962	44.5(±)	149 (15.5)**	N.A.	N.A.

* Incomplete; ** p=0.000 Chi-square test, (HP: Helicobacter pylori)

The mean age of patient group was 51.4(±15.3), female 61 (70.9%) and male 25 (29.1%). HP Stool Ag test result which is marker of active *H. pylori* infection was 35.1% (27/77). The serological results of *H. pylori* infections were as *H. pylori* IgG 82.1% (69/84) and *H. pylori* CagA-IgG 47.7% (31/65). There was no significant difference between autoimmune diseases for anti-CagA-IgG positivity (p>0.05). Interestingly, the frequency of active infection (HP stool Ag, 35.1%) was significantly higher in autoimmune patients when compared with the data of our microbiology laboratory (15.5%) for 2015 (p=0.000).

In our study, approximately half of the infected autoimmune patients were positive for anti-CagA IgG that it is similar to results obtained from the samples collected in Turkey. In spite of the fact that lacking control group, in a similar manner to the some literature our first findings suggest that active *H. pylori* infections rates are higher in patients with autoimmune diseases when compared with our laboratory data.

Keywords: *Helicobacter pylori*; autoimmune diseases; CagA; rheumatoid arthritis.

[P-0167]

[Tumor Immunology & Molecular Markers]

Analysis of myeloid-derived suppressor cells (MDSCs) from breast and colorectal cancer patients

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Introduction and Aim: The soluble factors from tumor microenvironment and chronic inflammation increase myelopoiesis and these circulating immature myeloid-derived suppressor cells (MDSC) can lead to tumorigenesis, metastasis and angiogenesis. This study investigated MDSC population immunophenotype and function in peripheral blood samples were collected from healthy donor, and patients with breast or colorectal cancer.

Methods: Peripheral blood samples were collected from healthy donors (n=10) and from newly diagnosed patients with breast cancer (n=11, early stage) or colorectal cancer (n=17). Blood samples were either used as whole blood or the leukocytes were separated according to their density with Ficoll-1077 and -1119 gradients. Myeloid cells were characterized with the markers related with monocytic and granulocytic MDSCs (CD45, CD11b, CD66b, CD14, CD33, CD125, CD14, CD16, HLA-DR, CD15) using multi-color flow cytometry. Dichlorodihydrofluorescein diacetate (DCFDA) and 4,5-diaminofluorescein diacetate (DAF-2DA) were used to measure ROS and NO production by MDSCs, respectively.

Results: In cancer patients, leukocyte counts were slightly higher than that of healthy individuals. $CD45^+CD11b^+CD125^-CD15^+CD66b^+$ polymorphonuclear granulocytes were increased in both cancer groups whereas $CD45^+CD11b^+CD125^-CD33^+CD14^+$ monocytic cells displayed a slight increase in colorectal cancer. Especially, both the percentage and number of granulocytic MDSCs ($CD66b^+CD33$hi in $CD11bhiCD125^-CD45^+$) was increased in cancer patients. These cells were both found in low and high density gradients however a clear sub-population was only observed in patient blood samples separated with 1077 low-density gradient. In the patient samples, monocytic cells appeared to harbor $CD66b^+$ neutrophil marker. By using these multi-color staining panel, MDSCs were successfully isolated with high purity (>98%) by FACS. Granulocytic subpopulation was also positive for CD14 and HLA-DR. Patient low-density neutrophils possessed lesser capacity to produce ROS than their high-density counterparts. These cells also demonstrated NO production as comparable to that of monocytes.

Conclusion: In breast cancer and colorectal cancer patients, CD66b and CD33 serves as useful markers to discriminate immature granulocytes and monocytes. Additionally, several myeloid cell markers and functional assets of these cells must be included in the staining panel to confirm MDSC subsets. These markers are useful for the purification of MDSCs to be used in functional T cell suppression assays.

This project is being supported by TUBITAK under the "European Cooperation in Science and Technology (COST) Project, BM1404 "European Network of Investigators Triggering Exploratory Research on Myeloid Regulatory Cells (Mye-EUNITER)".

Keywords: Myeloid-derived suppressor cells (MDSC); breast cancer; colorectal cancer.

[P-0169]

[Tumor Immunology & Molecular Markers]

Differential distribution and enrichment of myeloid cells upon ischemia/reperfusion (I/R) injury in experimental breast cancer

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Introduction and Aim: Ischemia reperfusion (I/R) injury may be commonly experienced regionally and/or in the extremities as a result of surgical operations including radical removal of breast tumors. Since both I/R injury and cancer are potent inducers of myelopoiesis, this study aims to determine distribution and levels of monocytic, granulocytic myeloid cells, and macrophages in healthy and tumor-bearing mice under the influence of short- and long-term of I/R.

Methods: Murine mammary carcinoma, 4T1 cells, were subcutaneously implanted to the left inguinal breast tissue in Balb/c mice. When the tumors reached to ~5 mm diameter, left hind limbs of the animals were ligated with orthodontic rubber bands for 90 min of ischemia followed by 3 and 7 days of reperfusion. The animals were euthanized; hind limbs, blood, spleen, liver and tumor tissues were harvested. The change in tumor size and number of metastases and myeloid cell infiltration in reperfused limbs were determined by macroscopic and histopathological evaluation. Myeloid cells were characterized by expression of surface molecules (CD45, CD11b, Gr1, Ly6G, Ly6C and F4/80) using multi-color flow cytometry.

Results: A great amount of myeloid cells infiltrated the reperfused limbs decreasing the peripheral blood leukocyte counts especially in control mice. Alternatively, the myeloid cell counts of tumor-bearing mice that already had leukocytosis were less affected by I/R even though histopathological evaluation of their reperfused limbs showed tremendous amount of myeloid cells. Generally, the number of total myeloid CD45+CD11b+Gr1+ cells 3 days after I/R in peripheral blood, and liver and tumor tissues was decreased while on day 7, their numbers were recovered potentially due to an increase in myelopoiesis. Subpopulations of CD11b+Gr1+ myeloid cells, i.e. granulocytic Ly6G+Ly6C+ and monocytic Ly6G-Ly6C+, followed the same trend. On day 7, the limbs became more severely infiltrated. Accordingly, in the tumor-bearing mice, peripheral blood and spleen were highly populated by myeloid cells. On day 3, the levels of myeloid cells, including monocytic and granulocytic cells and CD11b+Gr1-F4/80+ macrophages, were found to be decreased in the liver and tumors; this was followed by a restoration on day 7. Under the influence of I/R, tumor growth was provoked.

Conclusion: Following I/R injury in tumor-bearing hosts, spleen serves as a central organ for extramedullary hematopoiesis that accelerates myeloid cells' production, restores their level in the circulation but unfavorably enhances tumor growth.

Keywords: Ischemia/reperfusion injury; myeloid cells; breast cancer.

[P-0170]

[Cellular and Molecular Targets in Rheumatoid Arthritis and Autoimmune/Autoinflammatory Diseases]

Comparison of various antinuclear immunofluorescence antibody screening patterns and patients' neutrophil/lymphocyte ratio simultaneously

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Aim: Antinuclear antibodies (ANA) that bind to intracellular antigens are a distinctive feature of systemic autoimmune diseases, but also can be seen in other situations such as cancer and infections. It is claimed that the neutrophil lymphocyte ratio (NLR) is a potential marker for assessing inflammation. The relationship between patterns of antinuclear immunofluorescence antibody and NLR has not been investigated yet. In this study, we aimed to establish potential relationship among NLR and ANA immunofluorescence patterns.

Material and Method: In a retrospective manner (December 2014 - February 2016), a total of 218 adult patients with rheumatologic diseases that indirect immunofluorescence ANA test were positive and have CBC test result in same day were included the study as case group randomly. According to ANA immunofluorescence patterns, patients divided to subgroups (Table 1). Inclusion criteria for control group were: no diagnosed with autoimmune diseases, ANA test were negative and have CBC test result in same day with ANA test. CBC test was performed by Mindray cell counter (BC 6800) according to manufacturer's directions and sera samples were screened for ANA by indirect immunofluorescence (IIF) in Hep-20 cells (Euroimmun).

Table 1. Comparison of age, gender and NLR values between control and various IIF-ANA patterns

Study Groups	Age			Gender		NLR	
	N	Mean (±SD)	P*	Female n (%)	p*	Mean (±SD)	p*
Control Group (ANA negative)	30	52,5 (14,6)		21 (70.0)		3,08 (2,74)	
Case Groups (ANA positive) IIF Patterns							
Speckled granuler	35	49,9 (17,6)	0,521	32 (91.4)	0,026	3,97 (7,13)	0,498
Centromer	31	54,7 (11,9)	0,514	31 (100)	0,001	3,07 (3,38)	0,989
Nuclear Dots	30	54,7 (14.1)	0,562	30 (100)	0,002	1,93 (0,78)	0,033
Mix (Nucleolar cytoplasmic)	31	51,5 (16.4)	0,793	25 (100)	0,334	2,96 (3,08)	0,870
Nuclear Membrane	30	55,3 (14.6)	0,466	29 (96.7)	0,006	3,98 (3,97)	0,306
Homogeneus	29	47,2 (16.5)	0,194	19 (65.5)	0,713	3,37 (2,38)	0,663
Cytoplasmic Patterns	32	52,1 (14.0)	0,911	29 (90.6)	0,04	3,25 (3,93)	0,829

* Independent samples t-test.

Results: The results were summarized in Table 1. There was no significant difference between case and control groups for age (year). But there was significantly difference between gender and some IIF-ANA patterns. Interestingly, the neutrophil lymphocyte ratio was significantly lower in pattern of nuclear dots when compared with control group (p=0.033).

Conclusion: Although, we need well designed further studies, we think that the lower neutrophil/lymphocyte ratio may be associated with ANA patterns like 'nuclear dots'.

Keywords: Neutrophil/lymphocyte ratio; ANA patterns; nuclear dots; autoimmune diseases; IIF; indirect immunofluorescence.

[P-0171]

[Novel Discoveries in the Innate Immune System]

Immunosuppressive effects of synthetic oligodeoxynucleotide A151 on inflammasome activation

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Pathogen/Danger associated molecular patterns (P/DAMPs) are triggers for inflammasome activation that lead to caspase-1 (canonical pathway) or caspase-11/4 (non-canonical pathway) mediated production of IL-1b and/or IL-18 thereby contribute to inflammation and pyroptosis. NLRP1, NLRP3, NLRC4 and AIM-2 are some of several inflammasome activation pathways that have been described. Although it is a necessary course of response in healthy individuals, excessive inflammasome activation has been associated with the pathogenesis of Alzheimer's, gout disease, type-2 diabetes, atherosclerosis, and obesity; obligating the development of therapeutic agents that target distinct pathways of activation. Synthetic oligodeoxynucleotide (A151 ODN) expressing immunosuppressive TTAGGG motifs has previously been shown to ameliorate various inflammatory responses.

In this study, we induced the activation of NLRP3, NLRC4, and AIM-2 inflammasome activation in THP-1 monocytic cell line and subsequently treated with A151 ODN. Our results suggest, based on the suppression of IL-1β secretion and active caspase-1 formation that the activation of NLRC-4 and AIM-2 dependent but not NLRP3 dependent inflammasome pathways could be efficiently down-modulated following A151 treatment. These results suggest that suppressive ODNs could find therapeutic applications as potential inflammasome inhibitors in the clinic.

Keywords: Inflammasome; oligodeoxynucleotide; A151.

[P-0172]

[Novel Discoveries in the Innate Immune System]

Commensal bacteria-derived membrane vesicles as novel immunomodulatory therapeutic agents

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Gram negative pathogenic bacteria constitutively secrete membrane vesicles (MVs) that are enriched in TLR ligands and thus contribute to immune activation. Given the importance of microbiota as regulators of immune homeostasis, we aimed to assess the immunomodulatory properties of MVs secreted by 3 different human commensal lactobacilli isolates in comparison to *Escherichia coli (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 and 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 vesicles in an antigen independent chronic inflammation model, MVs were tested in a CCl4-induced liver fibrosis model. Results showed that, MV treated groups had decreased αSMA expression and ALT levels when compared to untreated fibrotic group. To determine the *in vivo* biodistribution of MVs, CFSE labeled vesicles were injected i.p. followed by their quantitation from spleen, lymph nodes, bone marrow or the peritoneal cavity 4h or 24h post-injection. Preliminary results showed that, MVs were mostly internalized by PEC after 4h and CFSE signal decreased within 24h. Moreover, the cells that internalized CFSE labeled MVs were found to express Ly-6G, suggestive of neutrophil-like phenotype. These results indicate that human commensal bacteria-derived MVs can have powerful immunomodulatory effects and can have potential therapeutic applications as novel anti-inflammatory agents.

Keywords: Commensal bacteria; membrane vesicles; immunomodulatory.

[P-0173]

[Novel Discoveries in the Innate Immune System]

Adenosine regulation of danger signaling

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Danger poses itself through different molecular signatures such as lipopolysaccharides, nucleic acids and lipoproteins with endogenous and exogenous origin, released after infections, injury and immunometabolic diseases. These signatures are sensed by both cell surface and intracellular/endosomal receptors, triggering MyD88/NFkappaB-associated signaling or TRIF/Type I interferon-associated signaling. The mode of recognition and the pathways induced significantly influence the amplitude and the onset of the immune response. Metabolic and immune related activities converge as main triggers of adenosine accumulation in extracellular space, which makes adenosine receptors important targets for inflammatory and metabolic diseases. Adenosine is known to modulate immunological activities during chronic viral infection, cancer progression and immunometabolic diseases. However,

how adenosine regulates different modalities of danger sensing is poorly understood. Here we show that almost all danger modalities increase the expression of adenosine A2A and A2B receptors. However, adenosine selectively and strongly suppresses MyD88/NFkappaB-associated signaling while sparing the expression of TRIF/Type I interferon-associated signaling outcomes such as IP-10. Adenosine crosstalk with especially cell-surface TLR ligands drastically increased anti-inflammatory IL-10, suggesting the existence of spatio-temporal effects on adenosine regulation of danger signaling. Overall our results indicate that there is a selective modulation rather than general suppression of immune responses by adenosine. Our results have important implications for developing combinatorial approaches to target adenosine and danger signaling to cure immune-related diseases. (This work is supported by grants from The Scientific and Technological Research Council of Turkey-TUBITAK- project no: 214S659, 115C062 and 115Z170).

Keywords: Adenosine signalling; TLR and danger signalling; immunoregulation.

[P-0175]

[Cellular and Molecular Targets in Rheumatoid Arthritis and Autoimmune/Autoinflammatory Diseases]

Altered isotope charge distribution of acetylcholine neurotransmitter and Myasthenia gravis

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Introduction: Acetylcholine (ACh) is a central neurotransmitter that is used for signal transmission among neurons. The neuromuscular junction releases ACh from the motor nerve terminal in discrete packages (quanta). The ACh quanta diffuse across the synaptic cleft and bind to receptors on the muscle end-plate membrane. Stimulation of the motor nerve releases many ACh quanta that depolarize the muscle end-plate region causing muscle contraction. Similarly, for signal transmission in neurons, a neurotransmitter must bind to its receptor in order to produce an action potential. It is known that in Myasthenia Gravis (MG), autoantibodies could block these bindings by attaching to the membrane and reducing the concentration AChR on the muscle end-plate membrane. In this study we propose a new approach to the treatment of MG, via modulation of molecular interaction between ACh and its receptor (AChR).

Methods: Quantum mechanical model allows us to investigate the geometries and energies of biomolecules. This model also provides us with electronic and vibrational spectra, electron and proton affinities and dipole moment. For a quantum mechanical modeling, a molecular system is an assembly and the properties of this assembly could be predicted by a wave function and could be solved by the Schrödinger equation with Born-Oppenheimer approximation. In this approximation, our group used the adiabatic Hamiltonian and obtained the average electronic energy of the molecule through Density Functional Theory (DFT) modeling. We also calculated the electrostatic interaction between neurotransmitter and its receptor.

Results: We have calculated the highest occupied molecular orbital and lowest unoccupied molecular orbital energies for ACh. Our results showed that with isotope-switching, some of the peak positions in the infrared spectra of ACh with 3 Deuterium and one O17 isotope were changed, but electronic energy calculations for the whole molecule requires further studies. Our results showed the dielectric constant of the synapse can be decreased for the attraction force to increase linearly.

Conclusion: In this study we suggest that if an atom on a ligand (i.e. a neurotransmitter) is replaced with its isotope, it may cause charge redistribution which could alter the binding between the ligand and its receptor. Hence this suggests that with replacement of molecules atoms with their isotopes in any biologically important ligand could possibly alter the ligand's affinity towards its corresponding receptor. We envision a wide array of applications of this approach in modern medicine.

Keywords: Acetylcholine, autoimmunity; myasthenia gravis; deuterium; isotope-switch; vibrational spectra.

[P-0177]

[Immunodeficiencies: Emerging Molecular & Cellular Therapies. Remembering Prof. Isıl BARLAN]

Determination of mitogen-triggered proliferation responses of thalassemia major patients treated with bone marrow transplantation

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Introduction: Thalassemia major is a common genetic disorder caused by gene mutations which encode the globin chains of the hemoglobin molecule. Thalassemia major patients present with severe hemolytic anemia, impaired organs and have increased risk for infections due to deficient immune responses. The only possible way of permanent treatment for thalassemia major is bone marrow transplantation. Determination of cellular responses are required for timing of vaccination after bone marrow transplantation. Carboxy fluorescein succinimidyl diester (CFSE) dilution is a flow cytometric approach widely used in measurement of proliferative capacity.

Aim: The aim of this study is the investigation of mitogen-triggered proliferative responses of pediatric bone marrow transplant recipients with thalassemia major.

Methods: Peripheral blood mononuclear cells were isolated from freshly heparinized blood samples of thalassemia major patients after 1 year of bone marrow transplantation, and also from healthy subjects. Cells were stained with CFSE and stimulated with polyclonal activators; anti-CD2, -CD3 and -CD28 (CD-mix) as well as phytohemaglutinin (PHA), and total, $CD4^+$ and $CD19^+$ lymphocyte proliferation levels were investigated on day +5 of cell culture.

Results: Cut-off values evaluated from healthy controls (n=20) were used for comparative evaluation of proliferative capacity of bone marrow recipients (n=23). Accordingly, 20 patients had normal proliferation values of total PBMCs, $CD4^+$ T cells and $CD19^+$ B cells, with no statistical significance when

compared to values of healthy controls. 3 patients were non-responsive to mitogens at various degrees. Of them, 1 was non-responsive to PHA, 1 was non responsive to CD-mix and 1 was non-responsive to both mitogens.

Discussion: Our results reveal re-gaining of proliferative capacity in thalassemia major patients after 1 year of bone marrow transplantation, which may be adequate for determination of vaccination time points. CFSE dilution by flow cytometry becomes a conformable tool for determining success of bone marrow transplant in immunological aspect.

Keywords: Thalassemia major; mitogen; cell proliferation; flow cytometry.

[P-0180]

[Inflammation and Diseases]

Evaluation of the pregnancy-associated protein A levels in patients with Hashimoto's thyroiditis

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Pregnancy Associated Protein-A (PAP-A) secreted from fibroblasts and vascular endothelial cells enhances accumulation of inflammatory cells and lipid laden macrophages thus causing progression of atherosclerosis. PAP-A also found to play role in atheroma rupture initiating the thrombosis.

The subclinical or treated thyroid disease is associated with increased cardiovascular risk. In order to determine the extent of this risk, and to explore whether the nature and/or treatment of thyroid disease are critical. Furthermore, markers of ongoing thrombosis are found to be better markers in early diagnosis of cardiovascular disease. In this pilot study we aimed to determine the relationships between serum PAP-A a proven indicator of cardiovascular disease in patients with HT.

Thirty patients with HT were recruited from outpatient clinics of Endocrinology and Metabolism department. All participants were selected from among women. Diagnosis of HT was based on fT4 level, TSH level, and thyroid antibodies positivity. The serum PAP-A concentration was quantified by enzyme-linked immunosorbent assays (ELISA). This is an experimental study. In this study, *in vitro* samples were investigated; any samples were not taken from patient.

The patients with HT were done with two sections based on clinical evidence, clinic HT (CHT, n=15) and subclinical HT (SHT, n=15). Relating to clinical situation, PAP-A levels were not found significantly different. But between CHT and SHT were found high correlation (22.4±6.1 ng/mL and 19.9±7.6 ng/mL respectively) (r=0.728, p>0.001).

The thyroid disease was associated with increased long-term cardiovascular risk despite restoration of euthyroidism. The mechanisms that mediate this risk are unclear but may not involve thyroid hormone abnormality. The use of PAP-A may be recommended for differentiation of cardiovascular risk with other endothelial dysfunction marker in patients with PD. But prospective studies should be planed about PAP-A.

Keywords: Pregnancy-associated protein A; Hashimoto's thyroiditis; cardiovascular risk.

[P-0181]

[Tumor Immunotherapy]

Targeting myeloid-derived suppressor cells (MDSCs) in pancreatic cancer by gemcitabine-loaded anti-VEGFR1 antibody-coated dendrimers carriers

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Introduction and Aim: Gemcitabine is an efficient chemotherapeutic for pancreatic cancer even though it has restricted success in disease-free survival. This drug has also capacity to eliminate myeloid derived suppressor cells (MDSCs) that favor tumor growth and metastasis. This study aims to investigate efficiency of originally synthesized dendrimers as targeted drug carrier system for gemcitabine targeting pancreatic tumors and tumor-induced MDSCs. Additionally, these dendrimers carriers were chemically linked to antibodies recognizing vascular endothelial growth factor(VEGFR)1 for the targeting of tumor cells and angiogenesis.

Methods: In poly (amidoamine) PAMAM dendrimer synthesis, following the successive alkylations, microwave assisted amidation by refluxing ester-terminated half-generation and excess ethylenediamine mixture. LPR method was used for purification. 1H NMR and ATR techniques were performed to verification of synthesis. Poly(ethylene glycol) (PEG) was used to prepare Gemcitabine HCl-dendrimer inclusion complexes. The amount of gemcitabine loaded was determined by HPLC. Cytotoxicity were evaluated by MTT to determine sub-toxic concentrations of dendrimers. Tumors were established in CD-1 nude mice with CFPAC-1 human pancreatic adenocarcinoma cells. When the tumors reached ~0.6 cm diameter, the mice were administered biweekly with physiologcal saline (SF), gemcitabine (GEM), gemcitabine-dendrimer inclusion complexes (Dend-GEM) or Dend-GEM with anti-VEGFR1 coating (Dend-GEM-VEGFR) for 3 weeks. Distribution and number of monocytic and granulocytic myeloid cells amongst bone marrow, spleen, peripheral blood and liver were evaluated by flow cytometry analysis of CD11b, Gr-1, Ly6G, Ly6C markers.

Results: In correlation with the size of the tumors, amount of CD11b+Gr-1+ myeloid cells, both CD11b+Ly6C+Ly6G- monocytic and CD11b+Ly6C+Ly6G+ granulocytic MDSCs, were increased especially in the spleen and blood. Treating with GEM or Dend-GEM or Dend-GEM-VEGFR reduced the size of the tumors. Notably, Dend-GEM-VEGFR was more efficacious in reducing the tumor burden. Even though the mice were administered with sub-toxic doses of gemcitabine, weight lost in mice receiving Dend-GEM complexes was higher. The percentage of myeloid cells in the bone marrow was considerably reduced in the chemotherapy treatment groups. Granulocytic myeloid cells were the most affected subtype when

gemcitabine was incorporated into dendrimers with or without anti-VEGFR1. Even though the percentage of myeloid cells was generally not different amongst the treatment modalities, in the Dend-GEM-VEGFR group, the number of CD11b+Gr-1+ myeloid cells, both monocytic and granulocytic MDSCs were substantially reduced in the circulation, liver and in bone marrow. The myeloid cells in the spleen was only slightly affected from the treatment

Conclusion: The gemcitabine-loaded anti-VEGFR1 antibody-coated dendrimers is an efficient preparation for targeting pancreatic cancers and MDSCs.

Keywords: Myeloid derived supressor cells (MDSC); immunotherapy; pancreatic cancer; gemcitabine; poly (amidoamine) PAMAM dendrimers.

[P-0182]

[Novel Discoveries in the Innate Immune System]

Myeloid cell boost induced by repeated surgery interferes with wound healing

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Introduction and Aim: Wound healing is divided into consecutive phases which are blood clotting, inflammation, tissue growth and tissue remodeling. Due to several complications, patients ought to undergo repeated surgical interventions. Since the elements of immune system, basically myeloid cells, play decisive roles in tissue repair, this study aims to assess the status of myeloid cells in repeated surgery model.

Methods: BALB/c mice were undergone laparotomy (LT) with ~3.5 cm sagittal cut through abdominal wall. The mice with single operation (once-LT) were sacrificed on days 2 and 7 days following laparotomy. A group of mice was re-operated (twice-LT) from the same wound trajectory whereas another group was undergone an additional surgery (thrice-LT) and these mice sacrificed 7 days following the last LT. Non-operated mice were control group. Peripheral blood, liver, spleen and abdominal wall specimens were collected for macroscopic and histopathological evaluation. The distribution and number monocytic and granulocytic myeloid cells amongst these compartments were evaluated by CD11b, Gr-1, Ly6G, Ly6C flow cytometry. Epithelialization, fibroblastic activity, ulceration, neutrophil and lymphohistiocyte infiltration were scored in the LT area. Trichrome and myeloperoxidase immunohistochemistry were performed on specimens. The capacity of granulocytic cells to modulate T cell responses were assessed by CFSE assay. Production of reactive oxygen species (ROS) was determined with DCFDA.

Results: Tissue healing was apparent on day 2 whereas incomplete epithelialization and inflammation were still evidenced on day 7. As an early event, the number of leukocytes were decreased both in the spleen (especially CD11b+Ly6C+Ly6G- monocytes) and blood. On day 7, spleen became highly populated by CD11b+Ly6C+Ly6G+ granulocytes. The liver was the least affected compartment. Second LT provoked fibrosis and scar formation in the abdominal wound characterized with lymphohistiocytes and lack of neutrophils. Monocyte enrichment was apparent both in the spleen and liver, leukocytosis was sustained in the blood. Enlargement of spleen was observed at this stage whereas the additional hit by thrice-LT resulted in splenomegaly. Wound area became ulcerative and highly infiltrated by neutrophils. Neurtophilia was a result of purging of splenic leukocytes into the circulation. These CD11b+Ly6C+Ly6G+ (even the low-density granulocytes obtained from 1077 Ficoll gradient) more effectively supported T cell proliferation than control neutrophils. They had ROS production capacity.

Conclusion: According to our results, local inflammation triggered by repeated surgery results in chronic inflammation and outspreads to a systemic level. This leads to severe neutrophilia reinforced by the spleen that interferes with wound healing.

Keywords: Wound healing; myeloid derived suppressor cells (MDSCs); repeated surgery; chronic inflammation.

[P-0183]

[Inflammation and Diseases]

Soluble urokinase plasminogen activator receptor (suPAR) in the diagnosis of pulmonary embolism

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Objectives: Pulmonary embolism (PE) is a common and potentially life-threatening disorder. There is still difficulties diagnosis of PE because clinical parameters and laboratory tests are nonspecific. Urokinase-type plasminogen activator receptor (uPAR) is a membrane-linked protein found in several cell types, including immunologically active cells and vascular endothelial cells. The soluble form of uPAR (suPAR) can be generated when uPAR is cleaved from the surface of such cells during inflammatory. The goal of this study was to compare the suPAR levels between PE patients and healthy subjects and also investigate the value of suPAR in the diagnosis of PE.

Methods: Thirty patients diagnosed with acut PE using spiral computerized tomographic pulmonary angiography were included in the study. suPAR and D-dimer levels were measured the time of diagnosis. Twenty nine age and sex matched healthy subjects were chosen for the study. The suPARnostic ELISA Standard Kit (ViroGates A/S, Birkerød, Denmark, Code No. A001,) was used for the quantitative determination of suPAR levels in plasma samples.

Results: The PE group's demographic and clinical characteristics are shown in Table 1. Median (95% CI) suPAR level measured in the PE group was 6.4 (6.4-10.5) ng/mL, compared to 3.3 (2.9-4.2) ng/mL in the control group ($p<0.001$, Figure 1). suPAR levels were significantly higher in the patients with PE ($p<0.001$). Receiver operating characteristic (ROC) curve analysis was performed to determine cutoff thresholds in discriminating between PE and control group plasma suPAR levels. Area under the ROC for that purpose was 0.871 (95% CI; 0.776-0.965). A suPAR cutoff point in patients with PE >4.3 ng/mL had specificity and sensitivity of 83% and 82%, respectively (Figure 2). There was a statistically significant positive correlation between increased D-dimer and suPAR ($r=0.530$, $p=0.004$).

Conclusions: This study suggests that suPAR values may be a biomarker with good sensitivity and specificity for diagnosis of PE. However, large prospective studies are required to further demonstrate the diagnosis significance of these values.

Keywords: Pulmonary embolism; diagnosis; suPAR.

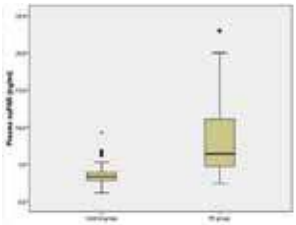

Figure 1. Plasma suPAR levels in the PE and control groups. *Horizontal lines represent the median of SCUBE1 levels for PE and control groups, 6.4 and 3.3 ng/mL, respectively.*

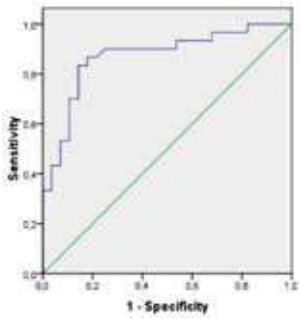

Figure 2. ROC curve of serum suPAR values for discriminating between the PE and control groups. *Solid line represents ROC curve and dashed lines represent the 95% CI of serum suPAR values. AUC= 0.871 (95% CI; 0.776-0.965). AUC= area under the curve; PE= pulmonary embolism; ROC= receiver operating characteristic.*

Table 1. PE group's demographic and clinical characteristics

Charateristic	n (%) or Mean (±SD)
Mean age (year)	61.73 (±17.4)
Sex Female Male	 14 (46.7) 16 (53.3)
Sign and symptoms Dyspnea Chest pain Syncope Hemoptysis DVT symptoms	 26 (86.7) 20 (66.7) 6 (20.0) 5 (16.7) 3 (10.0)
Phsical examination Heart rate (beats/min) Systolic BP (mm Hg) Diastolic BP (mm Hg)	 87 (13.0) 118.6 (21.6) 72.6 (10.8)
Surgery or trauma history	11 (36.6)
DVT	9 (33.3)
Comorbid disease	9 (33.3)
Thrombus location Main pulmonary artery (right or left) Lobar pulmonary arteries (right or left) Segmental and subsegmental pulmonary arteries (right or left)	 15 (50) 22 (73.3) 16 (53.3)
Pulmonary artery pressure (ECHO)	39.8 (17.4)
Wells score	4.6 (±1.10)
D-dimer leves	3060 (±4235)
CRP	43.5 (±39.5)

DVT: Deep vein thrombosis,ECHO: Echocardiographic, CRP: C-reactive protein.

[P-0185]

[Inflammation and Diseases]

The effect of fingolimod treatment on peripheral blood cell immunophenotypes in patients with multiple sclerosis

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Background: Multiple sclerosis (MS) is a chronic demyelinating disorder of the central nervous system characterized with inflammatory and neurodegenerative components. Fingolimod is a sphingosine-1-phosphate receptor modulator which sequesters lymphocytes in lymph nodes, preventing recurrent autoimmune relapses. The aim of this study was to investigate the mechanism of fingolimod treatment.

Methods: In this preliminary study, three patients with relapsing remitting multiple sclerosis (RRMS) were followed for six months with 3 visits (at baseline, before fingolimod treatment, 3 months, and 6 months after treatment). Blood samples were collected and Peripheral blood mononuclear cells (PBMCs) were prepared using whole blood lysis method to analyze $CD3^+$ T, $CD4^+$ T helper, $CD8^+$ cytotoxic T, $CD3^+CD4^+CD25^+$ Treg, $CD19^+$ B cells and $CD16^+CD56^+$ NK cells using flow cytometry. Freshly purified PBMCs were cultured ($2x10^6$ cells/ml) with phorbol 12-myristate 13-acetate (PMA), ionomycin and brefeldin A for 4h at 37 °C, 5% CO_2 for cytokine production. In order to determine intracytoplasmic cytokines anti-IFN gamma, IL17a, IL-10, IL-4, TNF-alpha, IL-9 monoclonal antibodies were used according to lymphocyte subsets by flow cytometry.

Results: Fingolimod depletes peripheral T helper, regulatory T and B cells and increases cytotoxic T and NK cells 3 and 6 months after treatment. Moreover, intracytoplasmic levels of all investigated cytokines were found to be reduced in each successive visit.

Discussion: Our results suggest that fingolimod may show its therapeutic effect through reduction of T helper and B cells, along with proinflammatory cytokines. Moreover, while fingolimod sequesters lymphocytes in lymph nodes, the innate immune system activity appears to be increased due to as yet unknown mechanisms.

Keywords: Multiple sclerosis; fingolimod; lymphocytes; flow cytometry.

[P-0188]

[Molecular Targets in Rheumatoid Arthritis and Autoimmune / Autoinflammatory Diseases]

The increased IL-22 levels in relapsing-remitting multiple sclerosis

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IL-22 is a proinflammatory cytokine produced by different cell groups such as Th22, Th17 and NK22 cells. The possible contribution of IL-22 to the inflammation in autoimmune diseases is supported by recent studies and several studies suggested that IL-22 may play a critical role in neurological diseases such as multiple sclerosis (MS), which is a chronic, inflammatory disease of central nervous system. However, the studies about the contribution of IL-22 in MS showed contradictory results, and the possible role of IL-22 is in MS still a question.

In our study, IL-22 levels in plasma and cell culture supernatant of relapsing-remitting (RR) MS patients were investigated. The heparinized peripheral blood samples were obtained from untreated (RR) MS, treated RR-MS and clinically isolated syndrome (CIS) patients and healthy donors. Plasma samples were collected and aliquoted and stored at -200 C. Peripheral blood mononuclear cells (PBMCs) were separated by gradient centrifugation. Supernatants were collected after PBMCs were cultured with IL-2, IL-10, IL-12, IL-27 and without any cytokine addition for 120 hours. IL-22 levels in plasma samples and supernatants were measured by using ELISA assay.

Plasma IL-22 levels of untreated RR-MS patients were elevated compared to both healthy donors and patient groups. Similarly, higher IL-22 levels of untreated RR-MS patients than healthy donors and other patient groups were also observed in culture supernatants without any cytokine stimulation. Following IL-2 stimulation, IL-22 levels were increased in all donor groups whereas they were notably elevated in untreated RR-MS and CIS patients. However, the levels of IL-22 were increased only in untreated RR-MS patients compared to healthy subjects and other patient groups in response to IL-27 stimulation.

These findings support that the possible inflammatory role of IL-22 in RR-MS pathogenesis. Furthermore, in the presence of IL-2, increased IL-22 levels could not be downregulated in untreated RR-MS and CIS patients as well as healthy donors and treated RR-MS patients. However, the observation which IL-27 stimulation led to elevation in IL-22 levels of untreated RR-MS patients but not CIS patients, suggests that there are some differences in the pathogenesis of CIS and RR-MS.

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A possible association between E469K polymorphism of ICAM-1 gene and nonobstructive azoospermia in southern Turkey

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Intercellular adhesion molecule-1 (ICAM-1), a member of the large immunoglobulin superfamily of cell adhesion molecules, is a constituent component of the blood-testis barrier, and it plays a significant role in homeostasis of spermatogenesis. The E469K polymorphism in human ICAM-1 gene has been previously associated in the various inflammatory/ autoimmune disorders. However, the role of ICAM-1 E469K polymorphism in spermatogenesis remains unclear. The present study aims to understand the association between ICAM-1 E469K polymorphism and male infertility with non-obstructive azoospermia (NOA) patients within a group of men from Turkey. We included 111 infertile male with NOA and 114 fertile male as control subjects to the study. Genotyping was made by polymerase chain reaction- restriction fragment length polymorphism. The frequency of genotype and allele of ICAM-1 E469K was not significantly different between control and patients ($p>0.05$). This is the first study to investigate the role of ICAM-1 gene polymorphism in male infertility with non-obstructive azoospermia. We conclude that the E469K polymorphism of ICAM-1 is not a risk factor for a non-obstructive azoospermia in Turkish population.

Keywords: Male infertility; ICAM-1; E469K; polymorphism; azoospermia.