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Innate immunity, inflammation and human models of diseases.

Auto-inflammation/Autoimmunity, Cancer and Viral Infections

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ABSTRACT BOOK

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BEST POSTER PRESENTATION

Flávio Almeida Amaral

THE LONG PENTRAXIN PTX3 CONTRIBUTES TO JOINT INFLAMMATION IN GOUT BY FACILITATING THE PHAGOCYTOSIS OF MSU CRystals

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Gout is the most common form of inflammatory arthritis and it is caused by the deposition of monosodium urate (MSU) crystals in joints. The early events involved in acute gout attack are the contact and the phagocytosis of MSU by resident cells that culminate in the maturation and release of IL-1β through NLRP3 inflammasome assembly. However, the precise mechanisms in these initial steps are still poorly understood. Here we investigated the role of pentraxin 3 (PTX3), a pivotal component of the innate immune system, in gout. PTX3 is elevated in plasma and synovial fluid during human gout flares and there was a positive correlation with IL-1β in synovial fluid. Experimentally, the injection of MSU crystals in mice increased the amount of PTX3 in inflamed joint. PTX3-deficient mice showed reduced joint inflammation in response to MSU crystals as compared to WT mice, including reduction of neutrophil recruitment into the joint cavity and the amount of IL-1β and CXCL1 in periarticular tissue. Moreover, mice treated with IL-1Ra reduced the amount of PTX3 in MSU crystals-injected joint. The incubation of recombinant PTX3 (rPTX3) with whole blood enhanced the phagocytosis of MSU crystals by monocytes. Corroborating, rPTX3 amplified the MSU crystals-induced IL-1β release by peritoneal macrophages. A similar response was observed in human PBMCs-stimulated cells. Mechanistically, the actions of PTX3 in this context seem to be dependent on FcγRs. The increase of phagocytosis and IL-1β production by MSU crystals in the presence of rPTX3 is abolished in total FcγR- and FcγRIII-deficient cells. Moreover, FcγR−/− and FcγRIII−/− mice had decreased joint inflammation following the injection of MSU crystals. In conclusion, our results suggest that PTX3 is an important molecule during gout attack by facilitating the phagocytosis of MSU crystal that amplifies joint inflammation.

Key words
Gout, inflammation, PTX3, IL-1β
Introduction: The anticardiolipin antibodies (ACA) recognize cardiolipin, a major component of the inner membrane of mitochondria, but other anionic phospholipids: phosphatidylglycerol, phosphatidylinositol and phosphatidylserine. They are mainly associated with antiphospholipid syndrome (APS). The ACA has been reported in some infections. Their exact role in these cases is not completely understood. Few studies have investigated these antibodies in sepsis.

Objective: The objective of our work is to study the evolution of ACA in sepsis and prognostic values.

Patients and methods: This prospective study was conducted in an intensive care unit over a period of 12 months. Two samples were taken for each patient at the time of diagnosis of sepsis (H0) and thereafter on the second day of hospitalization (H48). The enzyme immunoassay, indirect-type ELISA was used for the ACA highlighting to the two types of immunoglobulins IgM and IgG (Biosystem®, Barcelona, Spain). The normal value is 12 IU/ml for both isotypes.

The SOFA score was calculated H0 and H48. We used SPSS version 11.0, and the Wilcoxon test software comparison nonparametric variables. It is considered statistically significant if p <0.05. We also used the Pearson test for studying correlations.

Results: We collected 36 septic patients. In our study, three women and one man of 36 were positive including two ACA IgG and two IgM. We noted a significant increase in IgG and IgM ACA between H0 and H48 P = 0.01 and P = 0.02, respectively. We found a correlation between IgG ACA H0 to H48 and SOFA (R = 0.46).

Conclusion: ACA may significantly increase in sepsis. They can be a contributing element to the management of septic patients. Their exact role in this disease remains to be determined. Further studies are needed to verify these results.
Tumour-derived microvesicles (TMVs) are small membrane fragments released by tumour cells during their life span. They have a important contribution in intercellular communication during tumour progression as they modulate biological activity of immune cells e.g. monocytes and macrophages.

In the present study, the role of TMVs in monocyte differentiation and polarization of macrophages was investigated. Monocyte-derived macrophages (MDM) were differentiated \textit{in vitro} in the presence of TMVs obtained from colon cancer Caco-2, SW620, LoVo, SW480 cell lines and analysed according to their morphology and biological functions as defined by cytokine secretion, reactive oxygen intermediate (ROI) production and cytotoxic activity against respective colon cancer cells. Monocytes differentiated with TMVs exhibited morphological and phenotypical characteristics of macrophages. An early contact of monocytes with TMVs (at first day of \textit{in vitro} differentiation) resulted in increased IL-10 secretion, low ROI production and low cytotoxicity against tumour cells. On the other hand, late contact of MDM with TMVs, stimulated MDM to significant TNF and IL-12 secretion, ROI production and enhanced cytotoxicity against tumour cells \textit{in vitro}. Biological activity, STATs phosphorylation and microRNA profiling of MDMs indicated differences in their polarization/activation status which may suggest mixed polarization type M1/M2 with the predominance of M2-like after early contact with TMVs and M1-like cells after late contact with TMVs.

In summary, macrophages activity (polarization status) may be influence by contact with not only tumour cells but also with TMVs, however final polarization status depends on the contact time, and vesicle “cargo”.

\textit{ROLE OF FOXP3 GENE POLYMORPHISM IN THE SUSCEPTIBILITY TO TUNISIAN ENDEMIC PEMPHIGUS FOLIACEUS}

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\textit{Objective:} Forkhead box P3 (FOXP3) is an essential and crucial transcription factor of regulatory T-cells. Genetic polymorphisms in the promoter region of \textit{FOXP3} gene may alter the gene expression level and, therefore, contribute to several autoimmune diseases susceptibility. We aimed to investigate the possible role of genetic variants of four SNPs (rs3761547, rs3761548, rs3761549 and rs2294021) and a (GT)\textsubscript{n} microsatellite located in \textit{FOXP3} gene in the susceptibility to Tunisian Pemphigus Foliaceus (PF).

\textit{Method:} A case-control study was conducted on 98 patients with different clinical features of PF
and 182 matched healthy controls using PCR-RFLP method.

**Results:** According to the epidemiodemographic features of the disease, patients were classified into two groups: an endemic group (n=33, mean age=31[18-48]) versus a sporadic one (n=65, mean age=36[19-84]). In the whole population, rs3761548, rs3761549 and rs2294021 were associated with the susceptibility to PF. Interestingly, significant differences of gene distributions between the two subgroups of patients were observed. In the endemic group, all associations observed in the whole population were maintained and reinforced and a new association was revealed with rs3761547; while in the sporadic group, only the association with rs3761549 was conserved. Further, the haplotype analysis showed that the GA-C-15-C risk haplotype was significantly much more expressed in PF patients and specially in the endemic group. The phenotype-genotype correlation revealed that the rs3761548>AA genotype was significantly correlated with the severity of the disease including Nickolsky sign, generalized form of the disease and the earliest age onset.

**Conclusion:** These results underline the particular genetic background of the Tunisian endemic PF and suggest the implication of FOXP3 gene in the susceptibility and the clinical course of the disease.

**BEST POSTER PRESENTATION**

Mariem Ben Jmâa

Genetic variants and mRNA expression of IL23R in Tunisia Pemphigus Foliaceus.

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**Objective:** Th17 are a newly described subsets of T lymphocyte which are implicated in several autoimmune diseases. Recent evident suggests that the acquirement of the pathogenic character of these cells is under the control of the IL23 cytokine. The aim of our study was to evaluate the expression of IL23R in skin biopsy of PF and the genetic interaction of 4 polymorphisms in IL23R gene with the susceptibility to Pemphigus Foliaceus (PF), a blistering autoimmune skin disease.

**Material and methods:** We investigated 4SNPs (rs1884444, rs7517847, rs11209026 and rs10889677) of the IL23 gene in a case-control study including 106 PF patients and 203 healthy controls using PCR-RFLP. Genotype distribution and allele frequency were analyzed statistically using χ2 test. The odds ratio (OR) and its 95% confidence interval (95% CI) were calculated for each allele to estimate the magnitude of association using online software SHEsis. The mRNA expression of IL23R was evaluated in 3 patients' specimens compared to 3 healthy controls, using the TaqMan detection system. For the relative quantification, data were analyzed by the ΔΔCt method and normalized to the average of housekeeping gene GAPDH. Statistical analysis was carried out using GraphPad software.

**Results:** Genotype and allele distribution analysis showed significant associations between PF susceptibility and the rs11209026. Indeed, the A allele was found to be a protective allele for the disease (p=0.002, OR=0.35, 95%CI [0.18-0.70]). Moreover, patients were found to have significantly lower proportion of genotype containing A allele (AA: 0%; AG:10.4%), when compared with the normal controls (AA: 2.6%; AG:21.2%). No association was observed with rs1884444, rs7517847 nor rs10889677. The transcriptomic analysis revealed an increased expression of IL23R in PF patients' biopsies (0.0040±0.0024) compared to healthy controls (0.0017±0.0014) but the difference was not statistically significant (p=0.45).

**Conclusion:** Our results support the contribution of IL23R gene in the susceptibility the PF and its'
pathogenesis. The clinical implications in respect to the possible role of this cytokine in the development of the disease should be deeply investigated in a larger population.

**BEST POSTER PRESENTATION**

Arinna Bertoni

CHARACTERIZATION OF A NOVEL CAPS KNOCK-IN MOUSE MODEL TO EXPLOIT NOVEL APPROACHES FOR THE MODULATION OF THE NLRP3 INFLAMMOSOME

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**Introduction**

Gain-of function mutations in NLRP3 gene can lead to its constitutive activation resulting in an uncontrolled IL-1β production causing cryopyrin associated periodic syndromes (CAPS), a group of rare autoimmune inflammatory diseases characterized by recurrent fever, systemic inflammation and skin rash. We developed a new NLRP3 mutant Knock In (KI) mouse model, associated to the most severe CAPS phenotype, CINCA disease with central nervous system disabilities. Proton pump inhibitors (PPIs), commonly used for the treatment of excessive acid secretion by gastric cells, have been found to have anti-inflammatory effects making it a promising drug in sepsis and other severe inflammatory disorders.

**Objectives**

- to increase the knowledge on the pathologic consequences of NLRP3 mutations through a CAPS KI mouse model;
- to understand the underlying molecular and regulatory mechanism of IL-1β secretion;
- to identify molecular targets for the treatment of cryopyrin/NLRP3 related disorders.

**Methods**

Phenotypic and Immunological characterization of KI mice was performed by flow cytometry; IL1β secretion from bone marrow derived dendritic cells (BMDCs) and peritoneal macrophages (PMs) was evaluated by ELISA.

We are carrying out a preliminary pre-clinical study to investigate the response of KI mice to PPIs, such as esomeprazole.

**Results**

We engineered N475K mutation in murine NLRP3 gene and obtained two specific phenotype: heterozygous KI and homozygous KI mice. Both KI CAPS models develop systemic inflammation with consequent delayed growth, splenomegaly, hepatomegaly and skin rash; but early mortality after birth and neurological abnormalities, as difficulties in walking, are unique features of homozygous KI mice. Immunological characterization of peripheral blood and lymphoid organs reveal a pivotal role of innate immune system in severe inflammation of these CAPS KI mice, although, as a possible consequence, we also noticed an aberrant adaptive response.

The kinetics of IL-1β secretion from BMDCs and PMs was faster in KI cells, reaching plateau after 6h of 100 ng/ml LPS exposure, as happens in CINCA patients. Brief exposure to ATP strongly induced IL-1β secretion by LPSactivated WT cells while failed to stimulate further IL-1β secretion by KI inflammatory cells. Lower doses of LPS triggered high levels of IL-1β secretion in KI cells indicating that the NLRP3 mutation reduce the threshold of activation and increase the stress
status of the cells. As in CAPS patients, we confirmed the deficient production of IL-1RA and IL-6 after stimulation with LPS: the stress signs led to protein synthesis inhibition downstream of IL-1β. Esomeprazole reduced in vitro secretion of IL-1β produced by BMDCs and PMs isolated from both CAPS mice models. Remarkably, also in vivo PPIs treatment have been shown highly efficacious in inhibition of IL-1β release in heterozygous KI mice when compared to not treated KI mice.

Conclusions
We developed a well-established mouse model for CAPS autoinflammatory syndrome. Given the importance of IL-1β and inflammasome in innate immunity, NLRP3 knock-in mice could be applied to test new therapeutic compounds. Moreover, serious and severe clinical phenotype of homozygous KI mice could be used to gain insights into the mechanisms associated to neurological defects.

BEST POSTER PRESENTATION
E.A. Blinova

CENTRAL AND EFFECTOR MEMORY T CELLS IN THE MODEL OF HOMEOSTATIC PROLIFERATION IN VITRO IN NORM AND RHEUMATOID ARTHRITIS
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Homeostatic proliferation (HP) is a T-cell proliferation process triggered by a signal from receptors for interleukin (IL)-7, -15 and a signal via TCR for naive T lymphocytes. During lymphopenia-induced proliferation it is occurred the expansion of clones with TCR that has an affinity to self-antigens, the conversion of naive T cell phenotype into memory-like T cells. Based on this, it is formed assumption that HP makes a contribution to the development of autoimmune diseases, including rheumatoid arthritis (RA). It has been shown that memory T cells prevail in patients with RA and among them auto-reactive clones detected.

The study included 14 patients with RA and 6 healthy donors (average 48 years in both groups). PBMCs isolated from heparinised peripheral blood and cultivated with or without IL-7 (50 ng/ml), IL-15 (50 ng/ml) and IL-7+IL-15 in common in 24-well plate in full medium (RPMI-1640, 10 % FCS) during 7 days. Before and after culturing cells were stained with monoclonal antibodies conjugated with fluorochromes against CD3, CD4, CD45RO, CD62L, CD197. Analysis of T cell phenotype was performed on flow cytometer FACS Canto II (BD, USA). Before culturing there was no significant differences in amount of CD4+ and CD8+ RO-positive cells and central memory (CD62L+) cells between healthy individuals and patients with RA. In norm, stimulation with IL-7 and IL-15 lead to proportional expansion of central and effector memory T cells, and there were no significant differences in amount of memory cells before and after culturing. In RA patients, it was found that the proportion of memory cells increase among cytotoxic T-lymphocytes and CD4+ central memory cells in response to IL-15 and to IL-7+IL- 15. Furthermore, the addition of IL-15 to PBMCs from patients with RA resulted in an increase of the number of CD62L+197+ and CD62L+197-memory cells among CD8+ lymphocytes, although these changes were like a trend (p=0.067). In the T-helper population IL-15 had no such influence. The role of CD8+ cells in the pathogenesis of rheumatoid arthritis is considered to be minimal. However, in the synovium, CD8+ lymphocytes was the most presented among IFN-γ-producing cells, and these cells are associated with the presence of the germinal centers. In the peripheral blood of patients with RA the population of
CD8+45RA-62L+ central memory cells increased, which may indicate violations of cytotoxic lymphocyte homeostasis and an enhanced migration of terminally differentiated cells to the site of inflammation.

Conclusions: Given the high concentration of IL-15 in peripheral blood of RA patients, our data support the changes in the homeostatic control of populations of memory cells in this group. Also, it can be assumed that the various subpopulations of memory T-cells of patients with RA have different ability to respond to IL-15 stimulation.

**BEST POSTER PRESENTATION**

Chillemi Antonella

LOSS OF FUNCTION MUTATIONS OF HUMAN CD73 (NT5E) GENE AND IMMUNE SYSTEM.

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CD73 is a surface ecto-S'-nucleotidase (NT5E) which converts AMP to adenosine (ADO). ADO is a key regulator of inflammation and immunity, in the latter case exerting suppressive effects. Mutations in NT5E determine a clinical picture characterized by calcification of joints and arteries (CALJA). These mutations cause functional impairments of the surface CD73 and of ADO production, which in turn alters the balance of inorganic pyrophosphate/phosphate, leading to tissue calcification.

The aim of the present work is to extend the analysis of two sisters presenting with CALJA (reported by St. Hilaire et al., NEJM, 2011), paying attention to the functions of leukocytes considering their limited ability to produce extracellular ADO. ADO is exploited by regulatory T and B lymphocytes and by myeloid-derived suppressor cells (MDSC) to inhibit immune responses. However, direct observation of the affected patients did not reveal the presence of autoimmune disease or cancer. Further, phenotype and composition of T, B, NK and myeloid populations did not show significant differences when compared to age (>70) and sexmatched controls.

As CD73 is reported to regulate Ig class switch recombination, B lymphocytes of the two patients were immortalized by Epstein-Barr virus (EBV). The lines derived were analyzed for their Ig repertoire and their capacity to release ATP to be taken up by ectoenzymes (CD39 and CD73) involved in ADO production. The dynamics of ATP release from EBV lines from patients were apparently unaltered, according to TIRF (total internal reflection fluorescence). The study was then extended to the ability to secrete tolerogenic cytokines, in order to identify surrogate systems exploited by CALJA patients to circumvent the ADO deficit.

The results of qualitative and quantitative assays revealed no substantial variations in the panel of cytokines, except for IL-10, a cytokine of known tolerogenic function. The clinical symptoms of the patients prompted us to search for candidate substances linking immunity and osteogenesis. One of these was osteopontin (OPN), a pro-inflammatory cytokine reported to control calcium dynamics. The results indicate that B cells derived from the patients showed impaired release of OPN. This function was restored after transfecting cells with a wild type NT5E gene. The present finding may prove of therapeutic relevance for CALJA patients and at the same time may help in defining new pathways to circumvent an impaired extracellular production of ADO.
IL-17 POLARIZATION OF MAIT CELLS DERIVES FROM THE ACTIVATION OF TWO DIFFERENT PATHWAYS
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Background: Primary Sjogren Syndrome (pSS) is a chronic inflammatory disorder affecting exocrine glands. Both IL-23 and the downstream cytokines IL-17 and IL-22 are recognized as key players in the disease. Therefore, the identification of the cellular sources and inducers of IL-17 is crucial in the understanding of the drivers of inflammation in pSS. Mucosal-associated invariant T (MAIT) cells recognize antigens produced by the riboflavin biosynthesis pathway shared by many microbes and presented by the MHC class I-like molecule MR1. Thus, their activation occurs in an innate-like fashion and determines activation of effector mechanisms, such as cytokine production and cytosis.

Objectives: Recently, MAIT cells have been implicated in the pathogenesis of autoimmune disorders and found expanded in salivary glands of pSS patients. Their expression of IL-7R and IL-23R makes them potentially involved in the pathogenesis of pSS.

Methods: Mononuclear cells from 16 patients with pSS and 14 individuals with non Sjogren secca Syndrome (nSS) were isolated from blood and salivary glands. Phenotype and cytokine profile expression of MAIT cells were evaluated by flow cytofluorimetry analysis upon an in vitro stimulation with recombinant IL-7, IL-23 and IL-18.

Results: Frequency of MAIT cells was reduced in peripheral blood but not in minor salivary glands of patients with pSS, compared to patients with nSS. In vitro stimulation of MAIT cells from pSS patients caused cytokine production which was dependent on priming with IL-7, IL-23 and IL-18. Particularly, IL-7 and IL-23 guarantee IL-17 polarization of MAIT cells by two different pathways triggered by STAT3 and ROR-γt, respectively.

Conclusions: Our preliminary results confirm a potential role for MAIT cells in pSS and, for the first time, demonstrate the existence of a link between their specific IL-7 and IL-23 driven activation and IL-17 polarization.
immunodeficiency caused by dominant negative mutations in signal transducer and activator of transcription 3 (STAT3) and characterized by dermatitis, recurrent infections, elevated serum IgE, poor post-surgical healing, and a variety of skeletal and connective tissue abnormalities. STAT3 is involved in widespread physiological processes and in AD-HIES roughly 1/4 of wild type STAT3 dimers remain and preserve some transcriptional function. Although the underlying genetic cause of the disease has been identified, how STAT3 deficiency leads to this phenotype, is not well known. Currently, treatment options are limited to controlling infections by antibiotics and antifungal therapies. The aim of this study was to investigate which of STAT3’s many functions are dis-regulated in AD-HIES, and where potential targets for therapy may lie.

Methods: We used skin fibroblasts (SF) from 3 AD-HIES patients and 3 normal volunteers. To evaluate potentially affected pathways, we utilized RNA- Seq and subsequent Gene Set Enrichment (GSEA) and pathway analysis (Broad Institute, Pathway Studio, GeneGo Metacore software). Endothelial Cell Tube Formation Assay, a mouse model of hind-limb ischemia and teratoma tumor formation from patientspecific induced pluripotent stem cells (iPSC) were used to assess ability of AD-HIES SFs to support angiogenesis.

Results: GSEA and pathway analysis showed deficiencies in signaling pathways linked to wound healing, extracellular matrix remodeling and angiogenesis including many targets of Hypoxia Inducible Factor 1α (HIF1α) (P values for enrichments < 0.001). Therefore, we hypothesized that AD-HIES SFs have impaired ability to support angiogenesis due to deficient Hif1α-dependent secretion of matrix proteases and growth factors. Indeed, AD-HIES SF secreted up to 5 times less matrix metalloprotease 1, 3, and 9, placental growth factor and fibroblast growth factors (Luminex Multiplex Immunoassay, n=3-9, P<0.05). Consistently, cell culture medium from AD-HIES SFs failed to promote assembly of endothelial cells into vessel tubes resulting in lower number of junctions, meshes, and total tubule length (n=6, P<0.005). Injection of AD-HIES fibroblasts was not able to stimulate recapillarization in mouse hind limp ischemia model of angiogenesis. Teratomas formed in mice from iPSC cells generated from AD-HIES skin fibroblasts exhibited decreased ability to grow due to deficient capillary formation consistent with angiogenesis defects. Stabilization of Hif1α in AD-HIES SFs by prolyl hydroxylase inhibitor dimethyl fumarate (DMF) restored expression of MMPs and angiogenic growth factors (qPCR, n=5, P<0.05). Consistently, DMF restored ability of AD-HIES fibroblasts to support angiogenesis in endothelial cells tube formation assay (n=12, P<0.05) and improved teratoma tumor growth from ADHIES iPSCs.

Conclusion: The study identifies pharmacological stabilization of Hif1α as a promising treatment strategy for AD-HIES abnormalities related to angiogenesis, connective tissues metabolism and wound healing. The study was supported by Intramural Research Programs of NHLBI and NIAID.

BEST POSTER PRESENTATION

Nesrine Elloumi

ATYPICAL NEUTROPHILS OXIDATIVE BURST IN SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS

Immunology Department, Habib Bourguiba Hospital, University of Sfax, Tunisia

Objective: activated neutrophils (PMN) use multiple non-oxidative and oxidative mechanisms such as the oxidative burst. Which can result in collateral damage of host tissues and participate
actively in the inflammatory state. thus detection of any aberrant oxidative response or altered
mechanisms could have a crucial role in the pathogenesis of systemic lupus erythematosus (SLE). In
this study aim to assess ROS production rate after stimulation with fMLP or TPA and to investigate
the oxidative profile in lupus derived PMN. For this, different markers of oxidative stress were
considered; Lipid peroxidation, protein oxidation and catalase activity

Methods: After fMLP and TPA neutrophils stimulation of 30 SLE patients and 23 healthy controls,
ROS production was evaluated using a chemiluminescence assay. Then, oxidative damages in
neutrophils lysates were assessed by measuring Free thiol groups level, carbonyl groups, MDA
level and the catalase activity.

Result: SLE patients neutrophils showed a significant increase in ROS production level compared to
controls. In fact their neutrophils lysates were characterized by a decreased level of MDA, high
levels of protein oxidation and an elevated catalase activity compared to controls neutrophils
lysates. Surprisingly, upon subset analysis according to the disease activity, PMN of patients in
active phase exhibited a lower ROS production and higher oxidative damages than those in
remission phase.

Conclusion: Our study confirms that SLE patients derived PMN displayed an atypical behavior in
the two phases of the disease. Thus the characterization of the oxidative response may be an
indicator to pursue the disease activity.

BEST POSTER PRESENTATION

Nesrine Elloumi

UP-REGULATED EXPRESSION OF TOLL-LIKE-RECEPTOR 4 IN RENAL AND SKIN LESIONS
IN LUPUS ERYTHEMATOSUS

Immunology Department, Habib Bourguiba Hospital, University of Sfax, Tunisia

Objective: Toll-like receptor 4 (TLR4), a bacterial Lipopolysaccharide sensor, is an essential
modulator of the innate immune response. It is expressed on both immune and non-immune cells
and recognizes a panel of endogenous molecules released from injured cells and that may
contribute to the cutaneous and renal manifestations during Lupus Erythematosus (LE). Studies
conducted in vivo provide experimental evidence on the functional role of TLR4 in Lupus Nephritis
(LN) but its implication in lupus skin disorders is still unknown. The purpose of this study is to
analyze the role of TLR 4 in the pathogenesis of LN and cutaneous lupus erythematosus more
precisely chronic (CLE) by evaluating its expression in renal and cutaneous biopsies of LE patients.

Methods: This study was carried out on 30 Renal biopsy specimens from LN patients compared to
11 healthy renal tissues from healthy subjects. Skin biopsies were obtained from 30 CLE patients
and 15 normal individuals. All biopsies were taken for immunohistochemical staining of TLR 4.

Results: A strong and diffuse TLR4 expression throughout the epidermis combined to a labeled
inflammatory infiltrate and an intense TLR4 expression in the dermis glands were observed in CLE
patients’ biopsies; while normal controls’ skin expressed weakly TLR4 in the basal layer of
epidermis and not at all in the dermis.

We also showed an increased and more intense TLR4 expression respectively in LN glomeruli and
tubules compared to normal controls where TLR4 expression was weak and rarely detected in
glomeruli, diffuse and weak in tubules. A significant difference in TLR4 expression between LN classes, both in glomeruli and tubules was observed.

Conclusion: Our results clearly show an up-regulation of TLR4 expression in the affected tissues of CLE and LN patients and highlight the critical role of TLR4 in the pathogenesis of cutaneous and renal disorders in LE.

POSTER PRESENTATION
Edgar Fernández-Malavé

DIFFERENTIAL TCR EXPRESSION REQUIREMENTS FOR THYMIC DEVELOPMENT OF EFFECTOR GD T CELLS REVEALED IN CD3 DEFICIENT MICE

Department of Immunology, School of Medicine, Complutense University, Madrid, Spain

The role of the T cell receptor (TCR) in the generation of effector T cell subsets in the thymus is unclear. Prevailing models suggest that TCR signal “strength”, which is highly dependent on surface TCR expression, is a major determinant of the generation of discrete subsets of T cells producing either IFN- or IL-17. CD3 and CD3 are the most closely related CD3 proteins of the TCR. In the mouse, CD3 is incorporated in mature and immature surface TCR isotypes (TCR and pre-TCR), while CD3 is a bona fide component only in the TCR. Intriguingly, despite CD3 being absent from the surface TCR, mice deficient for CD3 display reduced surface TCR expression on T cells. The underlying mechanism and its impact on effector T cell development remain unclear. To clarify these issues, we analyzed effector T cell differentiation in mice deficient for CD3 (DKO mice), and in a mouse line lacking both CD3 and CD3, in which expression of a human CD3 transgene (hDTg mice) rescues otherwise suppressed TCR expression and T cell development. Along the differentiation pathway of IFN- producing T cell effectors, DKO mice showed increased abundance of the CD27+CD122+NK1.1 (IFN- low) subset, but reduced CD27+CD122+NK1.1 (IFN- high) cells, a putative progeny of CD122+NK1.1 cells which have presumably received strong TCR signals.

Notably, CD3DKO had decreased survival times with increased bacterial load after cecal ligation and puncture (CLP). Of interest, the introduction of N-ras deficiency in DKO mice improved TCR expression but did not correct the affected development of IFN- + effectors. hDTg mice, which expressed almost two fold more surface TCR than DKO, exhibited a selective reduction of CD27+CCR6 (IFN- producing) T cells, but without accumulation of CD27+CD122+NK1.1 cells, and with a concomitant increase of the CD27 CCR6+ (IL-17-producing) subset. Collectively, our study supports the existence of distinct TCR expression/signaling requirements for effector T cell differentiation in the thymus, with an impact in pathophysiology. Furthermore, it suggests an unexpected role of intracellular CD3, potentially involving N-ras signaling, for functional TCR expression during T cell development.

BEST POSTER PRESENTATION
GABSI Amira

CD146 AND CD146S: ACTORS AND TARGETS OF SCLERODERMA
University TUNIS el Manar, Faculty of science of Tunis
Summary: Systemic Sclerosis is a connective tissue disease characterized by excessive fibrosis of the skin and organs and the presence of autoantibodies in the serum. Among the new potential targets, CD146, which is a component of the endothelial junction, involved in signaling. A soluble form (CD146s) is generated either by proteolytic cleavage of the membrane form or by alternative splicing. This form is detectable in human serum. Its serum concentration varies during pathologies related to vascular dysfunction.

A genetic pathway previously known for its role in embryonic development and cancer has been identified as an objective for systemic sclerosis, or scleroderma. This pathway is the WNT pathway. We recently discovered a subpopulation of T cells called Memory Effector T Cells, which express CD146 and have a greater potential for cells migration to the inflammation site and secretion of proinflammatory cytokines.

Objectives: The detection of auto-anti-CD146 antibodies in the sera of 50 Tunisian patients affects systemic scleroderma. The Study of the involvement of the WNT signaling pathway in scleroderma and finally the subpopulations of T cells in PBMCs of patients with autoimmune diseases.

Materials and methods: ELISA did the auto-anti-CD146 antibody assay, using Rh-CD146s. Immunohistochemistry on paraffin-embedded biopsies from WT and KOCD146 mice and RTPCR to demonstrate the distribution of T lymphocyte subpopulations in various autoimmune pathologies.

Results and conclusion: Decreased anti-CD146 autoantibody levels in TUNISIAN patients with scleroderma. The results of immunohistochemistry allow us to say that the WNT pathway plays an aggravating role in the pathology, and confirms that the animal model KOCD146 is a pro-inflammatory model RT-PCR are not yet discussed (we will do it as soon as possible and send you an updated abstract).

Key words: CD146, Scleroderma, autoimmune disease, HUVECs, vascular dysfunction

BEST POSTER PRESENTATION

Ewelina Grywalska

EVALUATION OF PD-1 AND PD-L1 IN PATIENTS WITH PRIMARY PROLIFERATIVE GLOMERULONEPHRITIS

Department of Clinical Immunology and Immunotherapy, Medical University of Lublin, Lublin, Poland

Introduction: Programmed death-1 (PD-1) is one of the most important inhibitory co-receptors. Studies show that the PD-1/PD-L1 pathway regulates the induction and maintenance of peripheral tolerance and protects tissues from attack in physiological conditions. Recent studies have shown association of PD-1/PD-L1 pathway with several diseases although, to date, no such studies have been performed for primary proliferative glomerulonephritis (PPG).

Aims: The aim of this study was to describe the frequencies of T and B lymphocytes expressing PD-1 and PD-L1 molecules in patients with PPG.

Material and methods: The expression of PD-1 and PD-L1 was analyzed using flow cytometry on T and B lymphocytes collected from 20 adults with newly diagnosed, untreated PPG. The control group consisted of 20
healthy age-matched persons.

Results:
We observed statistically significantly higher expression of PD-1 and PD-L1 on analyzed lymphocyte subpopulations in patients with PPG than in healthy individuals. Among T cells, the frequencies of PD-1+/CD4+ T cells in patients (mean SD: 31.54 ± 13.74%) were higher than in healthy controls (mean SD: 5.35 ± 1.54%, p<0.001). Moreover, higher frequencies of PD-1+/CD8+ T cells and PD-1+/CD19+ B lymphocytes in the study group than in healthy volunteers were observed (mean SD: 18.71 ± 10.37% vs. 3.6 ± 1.45%, p=0.015 and 12.07 ± 4.34% vs. 1.67 ± 0.84%, p=0.017, respectively). There were positive correlations between PD-1+/CD19+ and PD-L1+/CD19+ B cells, and the serum level of IgA (r=0.43, p=0.026, and r=0.52, p=0.031, respectively).

Conclusion:
High expression of PD-1 and PD-L1 on T and B cells could represent the hallmark of immune system reaction to chronic antigenic exposition in patients with PPG.

BEST POSTER PRESENTATION

Giuliana Guggino

MIR 106A, MIR 19A-B, MIR 20A AND MIR21A REGULATE Vγ9Vδ2 FUNCTIONS PARTICIPATING IN THE INFLAMMATORY RESPONSES OCCURRING IN RHEUMATOID ARTHRITIS

Dipartimento Biomedico di Medicina Interna e Specialistica, Sezione di Reumatologia, Università di Palermo

Background: miRNAs are non-coding RNAs which have significant roles in regulating gene expression. The miR17-92 cluster appears to be a key factor in the inflammatory pathways activated during RA. In this study we aimed to evaluate miR17-92 expression and functions in γδ T cell subsets in RA patients, γδ T cells, in fact produce proinflammatory cytokines such as IFN-γ, IL-6 and IL-8 that may contribute to the inflammatory responses in RA.

Methods: Heparinized peripheral blood from 10 early RA untreated patients and 10 healthy donors was obtained for this study. Polyclonal Vγ9Vδ2 T cell lines were generated first by magnetic isolation followed by sorting (FACSARia) and further analysed by flow cytometry to define their phenotype and their pattern of cytokine production. Expression analysis of miRNA17-92 cluster was performed by RT-PCR and after identification of relevant miRNA involved in RA, mRNA expression of miRNA target genes was studied.

Results: A remarkable change in the distribution of Vγ9Vδ2 T cell functional subsets with an expansion of effector subsets and a reduction of naive cells, was observed in the peripheral blood of RA patients, as compared to healthy donors, which were accompanied by modifications in proinflammatory cytokine expression. Particularly, TEM (effector memory) and TEMRA (effector memory terminally differentiated) cells resulted the major source of IL-6 and IL-8. A significant correlation between miRNA expression and levels of IL-6 and IL-8 was found. The comparative analysis of miRNA expression among Vγ9Vδ2 T cell subsets, between RA patients and healthy controls showed a downregulation of miR106a-5p and miR20a-5p and an upregulation of miR 21a-5p among Vy9Vδ2 TEM cells; a downregulation of miR19-3p among Vy9Vδ2 TEM and TcM (central
memory) cells was also found. This miRNA expression regulated IL-8 and IL-6 gene transcription contributing to the survival of the proinflammatory pool reducing the expression of the PDCD4 gene.

Conclusions: Our results provide evidence of a role of miR106a, miR19-3p, 20a and 21a in the regulation of Vγ9Vδ2 T cells function in RA patients and suggest the possibility that the miR17-92 family and γδ T cells participate to the pathogenesis of RA.

**BEST POSTER PRESENTATION**

Imen Ben-Mustapha

**NOVEL GENETIC DEFECTS UNDERLYING AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME IN A HIGHLY CONSANGUINEOUS POPULATION**

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Faculty of Medicine, Tunis, Tunisia

Autoimmune lymphoproliferative syndrome (ALPS) is a primary immunodeficiency disease due to impaired Fas-FasL apoptotic pathway. It is characterized by chronic lymphoproliferation leading to enlargement of the lymph nodes, spleen and/or liver, associated with autoimmune manifestations primarily directed against the hematopoietic system. Deleterious heterozygous germline mutations in the FAS (CD95) gene inherited in an autosomal dominant pattern and associated with preserved protein expression, are the most common cause of ALPS. Somatic FAS mutations, detected primarily in double negative T cells, are the second most common genetic etiology of this disease. Autosomal recessive (AR) form of ALPS caused by homozygous germline FAS mutations is very rare with only four described mutations.

Herein, we report the molecular and functional analysis of ALPS in a highly inbred North African population. We identified, in two severe ALPS patients, two novel homozygous mutations underlying rare splicing defects mechanisms in FAS gene. The first mutation breaks a branch point sequence (c.506-16A>G) and the second alters a regulatory exonic splicing site (c.538C>T). Both mutations induce the skipping of exon 6, which encodes the transmembrane domain of CD95, thus precluding normal Fas expression. Quantification of FAS mRNA by RQ-PCR analysis experiments revealed the presence of the full length Fas transcript including exon 6 in the control whereas it was completely absent in both patients.

Our findings expand the spectrum of homozygous mutations with lack of Fas expression and highlight the requirement of tight regulation of FAS exon 6 splicing for balanced alternative splicing.

On another hand and interestingly, we identified in two other unrelated consanguineous Tunisian families a novel form of ALPS characterized by homozygous FAS gene mutations in either intracellular (c.1017A>G) or extracellular (c.581C>T) domains associated with normal or residual Fas expression respectively. Both mutations were predicted to be damaging and were not found in 100 healthy control subjects ruling out the possibility of irrelevant polymorphisms. Moreover, they induced severe resistance to Fas-mediated cell death when compared to control cell lines. Interestingly, the asymptomatic heterozygous parents showed a significant apoptotic defect but milder compared to homozygous patients. This residual protein function might be sufficient to...
prevent the development of ALPS in heterozygous parents, although the underlying mechanisms remain to be fully understood.

Dominant-negative effect and haploinsufficiency are associated with heterozygous FAS mutations and predominate mainly in outbred human populations. Our findings further illustrate that families from areas with a high rate of consanguineous marriages are important not only for the discovery of novel disease-causing genes but also for the identification of novel forms of known primary immunodeficiencies.

POSTER PRESENTATION

Monireh Jahantigh

EFFECTS OF $\beta$ - 17 ESTRADIOL ON THE AMELIORATING POTENTIAL OF ALLOGENIC MESENCHYMAL STEM CELLS IN ANIMAL MODEL OF RHEUMATOID ARTHRITIS

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Introduction: Multiple findings suggested that allogeneic mesenchymal stem cells (MSCs) possess potent immunoregulatory and regenerative properties. However, inadequate migration of transplanted cells reaching to inflamed tissues has limited their therapeutic benefits. This study was set out to evaluate the therapeutic potential of $\beta$ - 17 estradiol (E2)-treated mesenchymal stem cells in ameliorating experimental model of rheumatoid arthritis (RA).

Methods: MSCs were isolated from bone marrow of rats and pulsed with E2 (0 or 100 μM) for 24 hours. RA was induced by injection of complete Freund’s adjuvant in male Wistar rats (8-10 weeks old). MSC therapy ($2^{nd}$—106/rat) was initiated at day 10 post immunization when the mice developed a disability score and repeated 7 days later. Animals were monitored for 30 consecutive days.

Results: Treatment with E2-treated MSCs significantly regressed hind paw swelling and the arthrogram score of inflamed joints of rats more profound than these parameters in rats treated with MSCs alone. Ex vivo stimulation of splenocytes showed a significant decrease in Th1 (IFN-β, IL-2, IL-12 and TNF- β) and th17 cytokines (IL-17 and IL-23) production in cells from MSC-treated group and a significant increase in anti-inflammatory IL-10 secretion, compared to cells from vehicle-treated group. Of note, E2-treated MSCs significantly reduced TNF- β and IL-17 and conversely, increased IL-10 production more prominent than the similar findings brought about by MSCs without treatment. Also, C-reactive protein (CRP), nitrite/nitrate concentrations of serum were significantly regressed in the sera of rats received E2-treated MSCs more than sera of rats received MSCs without treatment.

Discussion: Our findings suggest that conditioning of MSCs with E2 promotes more beneficial effects than MSCs alone in the treatment of RA.

Key words: Rheumatoid arthritis Mesenchymal stromal cell migration, $\beta$ - 17 estradiol.
THE EFFECT OF MONOCYTE CELLS EDUCATED WITH SUPERNATANT OF
MESENCHYMAL STEM CELLS IN AMELIORATING EXPERIMENTAL MODEL OF CHRONIC
ASTHMA

Department of Microbiology, Faculty of Veterinary medicine, Urmia University, Urmia, Iran

Introduction: Pervious data suggested that monocyte/macrophages can be educated by mesenchymal stem cells (MSCs) or their supernatants towards the alternative way that may reduce inflammation and could modulate immune responses. This study was set out to investigate the therapeutic potential of monocyte cells educated with the supernatant of mesenchymal stem cells in ameliorating experimental model of chronic asthma.

Methods: BALB/c mice were sensitized by i.p. injection of ovalbumin (OVA) and alum (on 0,14th, 21th days) and then were challenged intra-nasally with OVA (on 27th-81th days,3/weeks). On 74th day post initiation, mice received monocytes (1×106/200 μl). At the end bronchoalveolar lavage (BAL) and splenocytes of mice were isolated and examined.

Results: Th2 (IL-4, IL-5 and IL-13) and th17 cytokines (IL-17 and IL-23), BAL total leukocytes count and the percentage of neutrophils and eosinophils and serum OVA-specific IgE were increased in asthma group compared to the control mice. Treatment with educated monocytes significantly decreased inflammatory cell infiltration in BAL fluids of mice and. Moreover, serum OVA-specific IgE Th2 and th17 cytokines were significantly decreased in treatment mice. Nevertheless, anti-inflammatory IL-10 was increased in treatment group compared to control mice.

Discussion: Using this strategy a sufficient amount of autologous anti-inflammatory monocytes could be prepared within a few days in a simple feasible fashion for treatment of asthma.

POSTER PRESENTATION

Monireh Jahantigh

EFFECT OF SESAME (SESAMUM INDICUM L.) ON CHANGES MORPHOLOGY OF
PANCREAS IN STREPTOZOTOCIN INDUCED DIABETIC RAT.

Department of Microbiology, Faculty of Veterinary medicine, Urmia University, Urmia, Iran

Objective(s): Diabete describes a group of metabolic diseases in which decrease insulin secretion of the pancreas. Sesame (Sesamum indicum L.) oil contains lignin with antioxidant activity, vitamin E. It can also be resistant to lipid oxidation as an antioxidant to remove hydroxyl, proxy radicals. The aim of this study was to investigate the effect of extract of Sesame on pancreas morphology.

Materials and Methods: Thirty mature male Wistar rats were randomly divided into three groups, i.e., control (C), diabetic-control (DC), and sesame-treated diabetic rats (SD). Diabetes was induced by a single dose of streptozotocin (65 mg/kg; i.p). The animals were treated by a single intraperitoneal sesame extract injection (200 mg/kg b.w.) once daily for 6 weeks. The pancreas of rats were removed and fixed and after tissue processing stained with H&E for light microscopic investigations.

Results: Sesame extract caused a significant increase (p<0.05) in numbers of islets.
Conclusion: This study suggested that sesame extract has antioxidant effect by increasing numbers of islets.

BEST ORAL PRESENTATION

Marinos Kallikourdis

T CELL COSTIMULATION BLOCKADE BLUNTS PRESSURE OVERLOAD-INDUCED HEART FAILURE

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ABSTRACT:
Heart failure (HF) is a leading cause of mortality. Inflammation is implicated in HF, yet clinical trials targeting pro-inflammatory cytokines in HF were unsuccessful, possibly due to redundant functions of individual cytokines. Searching for better cardiac inflammation targets, we linked T cells with HF development in a mouse model of pathological cardiac hypertrophy and in human HF patients. T cell costimulation blockade, through FDA-approved rheumatoid arthritis drug abatacept, led to highly significant delay in progression and decreased severity of cardiac dysfunction in the mouse HF model. The therapeutic effect occurred via inhibition of activation and cardiac infiltration of T cells and macrophages, leading to reduced cardiomyocyte death. Treatment also induced production of anti-inflammatory cytokine interleukin-10 (IL-10). IL-10-deficient mice were refractory to treatment, whilst protection could be rescued by transfer of IL-10-sufficient B cells. These results suggest that T cell costimulation blockade could be therapeutically exploited as a possible HF treatment.

BEST ORAL PRESENTATION

N.Y. Knauer

EVALUATION OF TELOMERASE ACTIVITY IN MONONUCLEAR BLOOD CELLS OF PATIENTS WITH RHEUMATOID ARTHRITIS AND HEALTHY DONORS UPON CYTOKINE STIMULATION IN VITRO

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Background: IL-7 and IL-15 are important regulators of lymphocyte maintenance and survival. They also play key role as homeostatic factors during the process of homeostatic proliferation (HP), which can happen in case of lymphopenia. It is supposed that the process of HP can be important in pathogenesis of rheumatoid arthritis (RA), because it can probably leads to increasing of the number of autoreactive clones. It’s still unclear how the process of HP can affect the telomerase activity in lymphocytes in the case of RA. It was shown that both IL-7 and IL-15 can activate telomerase, however, in case of RA,
the telomerase activity sometimes happens to be lower. Moreover, telomeres in lymphocytes, granulocytes and even CD34+-progenitor cells among patients with RA are shortened in comparison with healthy volunteers. Consequently, evaluating the dynamics of telomerase activity in blood cells of patients with RA and healthy donors can be interesting.

Materials and methods: 5 patients with mild and high activity of RA, age 37 (29; 53), and 5 healthy volunteers, age 35 (27; 49), were included in this study. Peripheral blood mononuclear cells were extracted from heparinized blood, then cultivated with IL-7, IL-15, IL-7+IL-15, and IL2+aCD3 for a week.

Cell lysates (day 0-7) were obtained and used for evaluating of telomerase activity according to the modified protocol of RT-TRAP (telomere repeat amplification assay). To calculate the relative telomerase activity, tumor cell line was used as a reference.

Results: We found that individual profiles of telomerase activity in days 0-7 can vary quite widely. However, we found that enzyme activity in donor group without any stimulation on day 6 is significantly higher, than on day 0. In case of RA, the curve of telomerase activity was two-peak and there was significant difference between day 0 and days 3, 5, 6, 7. In group with IL-7 stimulation, it was found that telomerase activity is significantly higher on day 5 in donor group and days 2, 3 and 5,6 in RA group (two-phase curve). The enzyme activity in group with IL-15 stimulation in donor group on day 5 was significantly higher than on day 0, in RA group - on days 3,4 higher than on days 5,6. In group with IL-7+IL-15 stimulation it was found that telomerase activity on day 5 is higher than on day 0 in donor groups and activity on day 0 in RA group is lower than on days 2, 3 and 6, 7. Telomerase activity in case of stimulation with IL2+aCD3 on day 0 was lower than on days 3, 5, 6 in both groups. However we didn’t found any significant difference in activity profiles between group of RA and volunteers.

Summary: The telomerase activity profiles were found to vary both in donors and RA patients. It can demonstrate the reaction of different cell subtypes to different cytokines or involving some different mechanisms of telomerase activation in cells during the experiment. These results can be useful for understanding some molecular mechanisms of RA pathogenesis.

BEST POSTER PRESENTATION

Giulia Macchiarulo

POST-INFECTION MILLER FISHER’S SYNDROME: ATYPICAL PRESENTATION DUE TO A HYPOMORPHIC RAG 1 MUTATION

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Recombination-activating gene (RAG) 1 mutations present a varied spectrum of combined immunodeficiency in humans, recently wide spectrum of phenotypes associated with residual protein activity have been described. We report the case of a one-year-old male affected by hypomorphic RAG 1 gene mutation. He was born from unrelated parents and came to our attention for asthenia, hyporeactivity, drowsiness and intense pallor. Previous history reported a sepsis by Pseudomonas Aeruginosa and BCGite at the age of seven months. Neurological exam
revealed severe axial hypotonus, ataxia, generalised areflexia, progressive paralysis of cranial nerves and swallowing difficulties. For the deterioration of respiratory function he was transferred to the Intensive Care Unit and assisted with non-invasive ventilation in C-PAP. Microbiological tests were performed for differential diagnosis, HIV infection was ruled out. CMV viremia was found, so intravenous ganciclovir therapy was started with not clinical benefit and never completely clearing CMV infection. It was clinically diagnosed a Miller Fisher’s Syndrome, later supported by the assessment of cerebrospinal fluid (CSF) test that revealed increased protein level (185.54 mg/dl) with normal glucose and CSF white blood cell count, and electrodiagnostic features of demyelinating and axonal damage. In addition blood examination revealed a Coombs positive hemolytic anemia.

Immunological exams revealed marked CD4 penia (4% 80 cells/m3) with marked expansion of memory T CD4 cell subset, T CD8 cell was increased, T cell receptor (TCR) γδ+ T-cells was expanded (69% of CD3 T cells). Memory B cells were expanded with reduction of transitional B cells and hypergammaglobulinemia (IgG 1981 mg/dl IgM 209 mg/dl IgA 137 mg/dl).

In order to control the expansion of B autoreactive clones it was started therapy with Rituximab and plasmapheresis allowing a complete depletion of B cells and progressive improvement of neurological and respiratory function.

The evidence of persistent immunological alterations, CMV infection and neurological features led us to suspect a primary immunodeficiency, so genetic tests were performed revealing an homozygous hypomorphic RAG 1 mutation (c.368_369delAA).

Because of severe clinical presentation HLA allogeneic hematopoietic stem cells transplantation was proposed. The lack of HLA-compatible donor led us to perform haploidentical-HLA transplant from partially compatible family donor (patient’s father). Post-transplantation analysis of chimerism showed evidence of full engraftment.

Four months post-transplantation the patient experienced severe autoimmune hemolytic anemia which required treatment with Igev, Rituximab, plasmapheresis and multiple blood transfusions up to progressive resolution.

The persistence of autoimmunity led us to measure BAFF plasma level that resulted high, in literature dysregulation of BAFF is described to contribute to the persistence of autoreactive B cells.

Miller Fisher’s Syndrome is thought to result from an immune response to an antecedent infection that crossreacts with peripheral nerve components because of molecular mimicry, the end result is an acute polynuropathy.

In the last years an increase number of PID associated with atypical and predominant autoimmune symptoms are described, so this case suggest clinicians to suspect PID in case of early onset autoimmunity or poor response to conventional treatment and reinforces the notion of pleiotropic phenotypic spectrum in RAG deficiency.

**BEST POSTER PRESENTATION**

Najla Mekki
Immunodysregulation, Polyendocrinopathy and Enteropathy X-linked Syndrome (IPEX) is a severe and rare monogenic primary immunodeficiency. It is caused by mutations in FOXP3 gene which is the master gene of regulatory T cells (Tregs) required for maintaining self tolerance. Classical IPEX syndrome is characterized by early onset of multisystem autoimmune syndrome with three main clinical manifestations: severe autoimmune enteropathy, eczema and endocrinopathy (Type 1 Diabetes Mellitus (T1DM), Thyroiditis...). It evolves extremely rapidly and the clinical course is most often lethal. 

Herein, we report a nine years male infant who presented since age 2 years with Type 1 diabetes mellitus, severe auto-immune anemia, diarrhea, atopic dermatitis and hepatosplenomegaly. Neither bronchopulmonary symptoms nor candidiasis were observed. Anti-transglutaminase antibodies were negative and intestinal biopsy examination showed no atrophy with a marked intestinal lymphangiectasia. Immune investigations revealed positive ANAs, anti-LC1, anti-parietal cells, anti-β2-glycoprotein and anti-cardiolipin. Thyroid function remained normal (FT4 : 15,65 ng/l, TSH : 5,89 UI/ml) despite the presence of antithyroid antibodies. Because of the multi-system autoimmunity observed in this infant, an IPEX syndrome was suspected and further investigation was performed. Immunophenotypic studies of lymphocyte populations and in vitro T-cell proliferation to mitogens and antigens were normal. The patient exhibited a high level of IgG but normal level of IgA and IgM. Interestingly, the total number of lymphocytes and lymphocytes subpopulations were normal, particularly Tregs were 4.9 % of the total number. No increase in the proportion of T cells with activated phenotype (HLA-DR+). Furthermore, patient’s cDNA sequencing of FOXP3 demonstrated that patient carried the splice variant lacking exon 2 also found in normal controls. Surprisingly, no mutation was found in the coding sequence, the 3'UTR region and the polyadenylation signal of FOXP3.

To date, no clear phenotype/genotype correlations have been described for IPEX syndrome, but atypical or milder forms have been reported and long term survivors have been observed. Searching for other underlying genes mutations should be performed. Indeed, molecular basis including STAT1 and STAT3 GOF mutations have been proposed to cause IPEX-like syndrome.
sulphate protect the CXCR3 ligands against proteolytic processing by CD26. GAGs did not decrease the direct enzymatic activity of CD26. They probably protect chemokines against CD26-mediated cleavage through steric hindrance in the NH2-terminal chemokine domain. In addition, GAGs were shown to interfere with the crosstalk between CXCR3 and its chemokine ligands. The observation that GAGs did not alter CXCL10-induced T cell chemotaxis in vitro possibly results from a combination of protection against proteolytic cleavage and altered receptor interaction. No significant effect of specific CD26 inhibition was found on CXCL10-induced chemotaxis in vitro. However, treatment of mice with the CD26 inhibitor sitagliptin resulted in an enhanced CXCL10-induced lymphocyte influx into the joint. This study reveals a dual role for GAGs in modulating the biological activity of CXCR3 ligands and supports the general hypothesis that both GAGs and CD26 affect the in vivo chemokine function.

BEST POSTER PRESENTATION

Nezha Senhaji

PRDX2 up-regulation in inflammatory bowel disease: friend or foe?
Laboratory of Genetics and Molecular pathologies, Faculty of Medicine and Pharmacy of Casablanca,
Hassan II University, Morocco

Background: Inflammatory bowel diseases (IBD) are chronic multi-factorial inflammatory disorders. Accumulating investigations have provided compelling evidence that describe the interplay of a complex genetic landscape and inappropriate inflammatory response to intestinal microbes in disease etiopathogenesis, but still pose challenges in diagnostic practices.

Method: In this study, comparative proteomic analysis was conducted to identify disease specific proteins underlying IBD pathogenetic mechanisms. Total blood proteins of the IBD patients and healthy subjects were analyzed with one-dimensional electrophoresis; differentially expressed bands were excised and subjected to MALDI-TOF/TOF-MS/MS along with nLC-ESI-MS/MS analysis.

Results: PRDX2 and Hemoglobin-subunits proteins, which are closely involved in the response to oxidative stress, were identified. PRDX2 was selected for further validation using Western blot and RT-PCR. PRDX2 over-expression was restricted to the protein level within the membrane fraction, further suggesting posttranslational modifications.

Conclusion: Our findings demonstrate the implication of PRDX2 in IBD. Future studies are required to establish its functional role and to determine the clinical utility.

Key words: PRDX2; Hemoglobin-subunits; IBD

BEST POSTER PRESENTATION

Darya Nizheharodava

PDGF and IgM as potential biomarkers in multiple sclerosis stem cell therapy
Belarusian Medical Academy of Post-Graduate Education, Minsk, Republic of Belarus;
International Sakharov Environmental Institute of Belarusian State University, Minsk, Republic
Introduction. Current pathogenic therapy of multiple sclerosis (MS) assumes combination of the immunomodulation and the strategy of oligodendrocytes protection from the further degeneration and the strengthening of remyelination. In this study, the methodological approach to the assessment of neuroprotective effects of cell therapy in MS patients after autologous mesenchymal/stromal stem cells (MSC) infusion is proposed.

Objective. To determine the role of serum platelet-derived growth factor (PDGF), immunoglobulins (Ig) G, M, A and autoantibodies to myelin-oligodendrocyte glycoprotein (anti-MOG) levels as potential biomarkers of the regenerative effect after autologous MSC infusion in MS patients.

Materials and methods. Peripheral blood was obtained from relapse-remitting MS patients (n=8) with expanded disability status scale (EDSS) score median of 2.5 before and after intravenous infusion of autologous bone marrow-derived MSC (mean 1.55 (0.92-2.35) x 10^6 cells per kg). Sera were harvested at baseline and 10 days, 1, 3, 6, 9, 12 months after cell therapy and monitored using ELISA kits: Quantikine Human PDGF-BB (DBB00, «R&D systems»), Immunoscreen - G, M, A (A-8674, «Vector-Best») and anti-MOG antibody (AEA421Hu, «Cloud-Clone Corp.»). Optical coherent tomography (OCT) was performed using «fast retinal nerve fiber layer (RNFL) thickness» algorithm.

Results. Before cell therapy, the level of serum PDGF of MS patients did not significantly differ from that in healthy donors. During 1-3 months after MSC infusion, the decrease of PDGF was established (p<0,05). The degree of PDGF decrease correlated with serum level of IgM (Rs = 0,87; p<0,05). After 1 month of cell infusion, serum IgM and IgG were temporary elevated in MS patients (p<0,01) along with the tendency to the decrease of circulating immune complexes and without any significant changes in anti-MOG antibody level. RNFL monitoring in MS patients has revealed the thickness increase after 6 and 12 months following MSC infusion, respectively, in 56 % and 50 % of MS patients’ eyes that correlated with PDGF concentration (Rs = –0,57; p<0,05).

Conclusion. PDGF and serum IgM levels in MS patients may be used as potential biological markers characterizing the neuroprotective effect of cell therapy.

BEST POSTER PRESENTATION

M. Pellicciotta

THE ROLE OF NADPH OXIDASE IN THE REGULATION OF INTESTINAL HOMEOSTASIS: IMPLICATIONS FOR CHRONIC GRANULOMATOUS DISEASE

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Humanitas Clinical and Research Center, Rozzano, Italy

Chronic granulomatous disease (CGD) is a primary immunodeficiency disorder of phagocytes, due to defect in the NADPH enzyme, resulting in impaired killing of bacteria and fungi. X-linked CGD is the most common genetic subgroup. In addition to phagocytes, the enzyme is expressed also in lymphocytes but its functional implication is still poorly characterized. Patients with CGD suffer from severe infections and deregulated inflammation. In particular, the mechanisms underlying the abnormal response of the intestinal immune system remain unclear. Analysis of pro-inflammatory cytokines was performed through real time PCR after RNA extraction from gut
tissue. Lamina propria lymphoid cells isolated from the gut tissue and single cell suspension from spleen, MLN, Peyer’s Patches were characterized by flow cytometry. Faecal IgA levels were quantified by Elisa essay. OVA-specific Treg conversion was analyzed in vivo upon adoptive transfer of CD4+ OTII cells and oral antigen delivery. Gp91phox-/ murine model shows signs of intestinal inflammation underlined by increased tissue expression of pro-inflammatory cytokines such as IL-17, IL-6, IL-1beta, IL-10 and antimicrobial peptides. Treg cell frequency is reduced in knock out mice as well as the percentage of CD103+ DC, endowed with tolerogenic functions. In line with these results, preliminary analysis showed that Treg cell polarization was affected in gp91phox-/ mice, upon oral OVA administration. Moreover, preliminary analysis also showed defect in the IgA compartment of knock out mice, crucial to contain gut microbes and maintain intestinal homeostasis. Consistently, expression of the epithelial antimicrobial peptide Reg3gamma was elevated compared to wild-type mice. The results obtained so far indicate that evidence signs of an inflammatory environment and a deregulated intestinal homeostasis likely due to the contribution of different cell compartments.

**POSTER PRESENTATION**

Penco F.

**Monocytes proteomic profile of patients with different autoinflammatory diseases: an approach to identify new biomarkers**

Laboratorio di Immunologia delle Malattie Reumatiche, Pediatría Seconda, IRCCS Istituto G. Gaslini, Genova

**Introduction:** Autoinflammatory diseases are a group of inherited diseases characterized by early onset and systemic inflammation, often manifesting with unexplained fevers. These pathologies are usually caused by mutations in genes involved in the regulation of innate immune response with consequent inflammatory phenotype driven by activation of monocytes, macrophages and granulocytes. Part of this pathologies are however genetically undefined. 

**Objectives:** Our aim is to evaluate the differences in the expression of proteins or pathway in monocytes, untreated or treated with LPS, of patients with autoinflammatory diseases and healthy subjects in order to clusterize the different diseases and better characterize the genetically undefined pathologies.

**Methods:** Monocytes, purified form peripheral blood of patients and healthy subjects with CD14 microbeads positive selection, were collected and incubated for 4 hours with or without LPS stimulation. Cells were lysed and prepared to be analyzed at MS. The samples were processed by iST protocol and protein expression evaluated by an High Resolution/Mass Accuracy Liquid Chromatography Tandem Mass Spectrometry (HR/MA LC MS/MS). Each sample was run at least in triplicate. PCA analysis and Person’s correlation were used as quality control of experimental design, while the statistical analysis was performed with the Perseus software.

**Results:** We analyze the monocytes of patients with CAPS, TRAPS, FMF and IperIgD (each group compared with healthy subjects). We have identified about 4000 proteins of each 3500 are quantified by LFQ approach. PCA analysis and Person’s correlation show a good reproducibility of data and a good separation between the different groups. In synchronous way using a cluster analysis and heatmap, based on a machine learning protein selection, we observe a protein
signature specific for each group of pathology and for each condition of monocytes treatment. Conclusions: Here, we addressed how an high resolution proteomics approach could be used to better understand the biology of autoinflammatory diseases. The characterization of a broad spectrum of proteins and their interaction network will allow us to identify new biomarkers for the different pathologies and to better comprehend and recognize the genetically undefined disorders.

BEST POSTER PRESENTATION

Elena Pontarini

NK CELLS RECRUITMENT IN VIRAL INDUCED SIALOADENITIS PROVIDE AN EARLY VIRAL CONTROL BUT ARE DISPENSABLE FOR THE FORMATION OF TERTIARY LYMPHOID STRUCTURES.

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Background:
The organization of immune cell aggregates in Tertiary Lymphoid Structures (TLS) with ectopic germinal centres characterizes chronic inflammatory responses against several viral infections. The salivary glands (SGs) represent a permissive site for several sialotropic viruses whose persistence has been linked with the development of autoimmunity and Sjögren’s syndrome. Natural Killer (NK) cells play a key role in viral clearance but their contribution to the control of viral infection in the SG and the subsequent formation of TLS is unclear.

Aim:
We recently developed an inducible murine model of TLS formation upon Adenovirus (AdV) infection of the SG. The main goal of this study was to investigate the recruitment and functional relevance of NK cells in the early phases of the antiviral immune response and in TLS formation and maintenance.

Methods and results:
A luciferase-encoding AdV was delivered into SG via retro-cannulation of excretory ducts to induce sialoadenitis in wild-type C57Bl/6 mice. Flow cytometric analysis of CD45pos/NK1.1pos/CD3neg NK cells in digested SG showed that, upon AdV5 cannulation, NK cells number rapidly increased in the glandular parenchyma, as confirmed by immunofluorescence (IF) staining for NK1.1pos/CD3neg. Adoptive transfer experiments demonstrated that NK cells were actively recruited from peripheral blood, and underwent proliferation within the SG. Infiltrating NK cells expressed the natural cytotoxicity receptor NKp46, acquired cytotoxic potential with an increased degranulation potential assessed with CD107a functional assay, produced IFN-γ and inhibited viral replication (as assessed by luciferase activity in vivo and in vitro) in the acute phase of inflammatory responses. Nonetheless, the selective antibody-mediated systemic depletion of NK cells in murine SGs did not affect the number, organization and functionality of TLS evaluated by histology.

Conclusion:
These data demonstrate that, upon local viral infection, NK cells are actively recruited to the inflammatory site and are critically involved in the early immune control of AdV infection in the SG but are dispensable for the development and maintenance of TLS within SG.

**BEST POSTER PRESENTATION**

Rosita Rigoni

**UNRAVELING PATHOGENIC MECHANISMS UNDERLYING SKIN DEGENERATION IN OMENN SYNDROME**

Istituto di Ricerca Genetica e Biomedica, Milan Unit, CNR, Milan, Italy
Humanitas Clinical and Research Center, Rozzano, Italy

Skin degeneration represents a common clinical sign in several Primary immunodeficiencies (PID), including Omenn Syndrome (OS), a peculiar immunodeficiency caused by hypomorphic mutations in RAG genes leading to the generation of oligoclonal activated T cells. Specifically, OS patients present cutaneous manifestations including vitiligo, psoriasis and atopic dermatitis, representing the clinical hallmarks of the disease. However, the underlying pathogenesis still remains elusive. Here, we investigated the mechanisms of skin autoimmunity in Rag2R229Q mice, the OS murine counterpart, which develop spontaneously several cutaneous lesions. In particular, the skin compartment of Rag2R229Q mice is characterized by the lack of epidermal γδ T cells and, consequently, by the marked accumulation of dermal CD4+/CD8+ T cells producing IFNγ abnormally. Increased expression of T cell recruiting chemokines, cxcl9 and cxcl10, produced by keratinocytes sustained the lymphocytic infiltration in the inflamed skin of Rag2R229Q mice. In agreement, peripheral CD4+ T cells showed higher expression of skin homing receptors.

Interestingly, upregulation of different TLRs was observed in the mutant skin, suggestive of constant exposure to several microbial products. In agreement, Rag2R229Q mice showed an altered bacterial load and an augmented level of the antimicrobial peptide Cramp in the cutaneous tissue, indicating a barrier defect. In addition to T cells, the inflamed skin was enriched of granulocytes and natural killer (NK) cells. Overall, our results point to an alteration of the skin barrier integrity, resulting in keratinocyte activation and marked infiltration of immune cells in the dermis. In OS, leakage of gut-endothelial barrier and loss of tolerance to commensal microbes lead to the systemic dissemination of pathogenic Th1/Th17 T cells. Thus, further investigations are aimed to elucidate how commensal antigens and gut-derived pathogenic T cells, reprogrammed to express skin homing receptors could potentially modulate skin immunity, contributing to cutaneous degeneration in OS.

**POSTER PRESENTATION**

Alessandra Roberto

**UNCONVENTIONAL CD56DIMCD16– NK CELLS HAVE A PIVOTAL ROLE IN THE NK CELL RECOVERY AFTER HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION**

Laboratory of Translational Immunology, Humanitas Clinical and Research Center, Rozzano, Milan, Italy
Natural Killer (NK) cells are innate lymphocytes playing a key role in the host defence from the early weeks of immune reconstitution (IR) following allogeneic hematopoietic stem cell transplantation. Here, we analysed the NK cell IR in a cohort of 28 lymphoma patients who receive a T cell replete haploidentical hematopoietic stem cell transplant (haplo-HSCT) to reveal the mechanisms at the basis of the NK cell maturation process and of the graft versus tumor effect. Our finding demonstrate that a subset of unconventional CD56dimCD16− NK cells (uCD56dim) plays a key role in the NK cell IR and represents the main NK cell population expanded in the first weeks after haplo-HSCT. These uCD56dim cells are bona fide NK cells that are present at very low frequency under physiological conditions, express both activating and inhibitory NK cell receptors (NKP30, NKG2D, CD94, NKG2A, NKG2C and KIRs) and display cytotoxic capability. In the first three weeks following haplo-HSCT we found proliferating uCD56dim that outnumber all other NK cell subsets. These cells derive from the donor graft and precede the appearance of conventional CD56bright (cCD56bright) and CD56dim/CD16+ (cCD56dim) NK cell subsets. In vitro experiments confirmed that uCD56dim generate CD56bright cells upon IL-15 and IL-2 stimulation, thereby suggesting a new precursor-progeny relationship of NK cell ontogenesis occurring in the lymphopenic environment that is present following haplo-HSCT. Gene expression profiling revealed that uCD56dim are similar to cCD56dim NK cells in healthy donors, while develop a unique specific pattern in haplo-HSCT patients, sharing features with both cCD56bright and cCD56dim NK cell populations. Moreover, our phenotypic analyses combined with functional experiments showed that, similarly to cCD56dim NK cells, the subset of uCD56dim NK lymphocytes from patients early after haplo-HSCT are armed to kill tumor cell targets (they express Granzyme B and Perforin) but express high levels of the inhibitory receptor NKG2A and have poor cytotoxic capacity. Only at later time points these cells acquired cytotoxic capability. Overall, these data characterize the expansion of a specific subset of uCD56dim NK cells driving NK cell IR starting in the early weeks following haplo-HSCT. Furthermore, our findings shed light on those NK cell-mediated mechanisms that can be targeted to improve NK cell cytotoxicity against both residual tumor cell and recipient dendritic cells presenting host antigens to donor T cells, thus reducing the risk of tumor relapse and graft versus host diseases, respectively.

POSTER PRESENTATION

Schena Francesca

DEFECT OF ADAPTIVE IMMUNITY IN ADA2 DEFICIENCY PATIENTS

Dipartimento di Pediatria 2, Istituto Giannina Gaslini, Genova, Italy

Introduction

ADA2 Deficiency is a new autoinflammatory disease characterized by systemic vasculopathy and episodes of strokes. However, some patients can present mild immunodeficiency. This defect is due to a loss of function mutation of CECR1 gene, coding for Adenosine Deaminase 2 protein. This protein regulates the catabolism of extracellular adenosine, which we have shown is an important regulator of Class Switch Recombination in B lymphocytes.

Objectives

As some DADA2 patients show hypogammaglobulinemia and recurrent infections, we investigated the role of CECR1 mutation on adaptive immunity. Therefore we decided to characterize
Peripheral B and T lymphocytes of DADA2 patients to directly address whether ADA2 mutation affects B and T cell interaction.

Patients and Methods
12 patients carrying homozygous mutations in CECR1 were examined. They showed clinical history with livedo reticularis, fever, vasculitis and neurological symptoms. 2 patients presented hypogammaglobulinemia, whereas others 2 patients presented recurrent infections. We analyzed peripheral B and T cell phenotype by flow cytometry, CECR1 gene expression in B and T lymphocytes by qRT-PCR. B cells isolated from healthy donors and DADA2 patients have been cultured alone or in coculture with CD4+ T cells. In vitro B cell proliferation and differentiation to Immunoglobulin secreting cells in response to TLR9 agonist have been evaluated by CFSE dilution and ELISA assay. Moreover cytokines production from T cells has been evaluated.

Results
Flow cytometric analysis of peripheral blood showed a significant reduction in the pool of memory B cells (CD19+CD27+), in particular switched B cells, in DADA2 patients compared with age matched controls. Moreover we observed a significant decrease in CD4+ and CD8+ cells, whereas the T cells subpopulations are normally represented. Interestingly peripheral Follicular Helper T cells are significantly increased in DADA2 patients. Then we addressed B cell defect in DADA2 patients, focusing on the interaction between B and T cells, essential for generation of B cell memory and for T cell dependent response of B cells. To this end, we cocultured CD19+ B cells and CD4+ T cells, isolated from patients’ peripheral blood. Analysis of B cell proliferation and differentiation showed that the proliferation of mutated B cells and Igs secretion were much more reduced in the presence of patient’s T cells than with normal T cells. Moreover IFNγ, TNFα and IL17 production from T cells did not differ between DADA2 patients and controls, whereas the evaluation of IL4 and IL21 are ongoing.

Conclusions
Our findings suggest that CECR1 mutation could lead to a defect in B cell function and to an altered T cell help of B cells.

BEST POSTER PRESENTATION
Seninet. E

THE ROLE OF OXIDATIVE STRESS IN THE EVOLUTION OF PRIMARY BILIARY CIRRHOSIS
Department of Physiology & Cellular Biology , Saad Dahleb Blida-1 University, Algeria

Background
The liver is continually exposed to high antigenic loads of pathogens. Loss of tolerance against its own antigens, leading to autoimmune hepatitis (AIH) or primary biliary cirrhosis (PBC) This lymphotropism is responsible for activation and one B cell proliferation, which has as main consequence production of antibodies whose are more frequent AMA type M2 and ANA the purpose of this study is to evaluate the different auto-antibodies and their specificities in the diagnosis of PBC, thus evaluating the status of oxidative stress on this autoimmune disease in order to better understand their specificities in the diagnosis of PBC and in order to better understand their etiologies.

Patients & Méthodes
Thirty PBC patients (24 to 75 years) and 30 control subjects were studied. The first step was to identify the anti-smooth muscle (ASMA), antimitochondrial (AMA) and anti-nuclear (ANA)
autoantibodies by IF on triple substrates and HEp-2 and, AMA type M 2, M 4 and M 9 by Dot-blot, quantification of AMA M 2 by Luminex and IgG F-actin by ELISA. The second step involved the study of oxidative stress parameters Malondialdehyde (MDA) Glutathione reduced (GSH) and NOx (NO).

Results
There is a female predominance sex-ratio 14/1. The study of autoantibodies to the following results: 17 ASMA positive patients develop AIH type I, 30 patients are AMA positive 17 patients are positive ANA develop PBC, with 11 AMA M 9 positive patients who are in the early stage of PBC. It is observed that 17 patients are ASMA and AMA positive, indicating that they develop an overlap syndrome. The results of oxidative stress parameters: NO 42.5 ± 11.24; MDA 44.19 ± 11.86 mol / l; GSH 43.14 ± 0.067 mmol / l a significant difference (p<0.001) was observed between the patients and the control cases. Multiple correspondence analysis revealed a non-significant correlation between ASMA and AMA, and a significant correlation (p<0.001) between the AMA and ANA.

Conclusion
The research of the AMA by IFI remains the best technique for the diagnosis of PBC, however the search for AMA type M 4 and M 9 is obligatory in order to establish the state of evolution of the PBC. In the light of all the results obtained, it appears that there are correlations between the intensity of the autoantibodies and the markers of oxidative stress. Our results support that the AMA may be induced by the oxidative stress resulting in exposure of the immune system to both native and modified intracellular proteins.

Keywords
Primary biliary cirrhosis, Autoimmune hepatitis, Overlap syndrome, Oxidant Stress

POSTER PRESENTATION
Tatiana Spadoni

RITUXIMAB TREATMENT REDUCED ACTIVATION OF FIBROBLASTS ISOLATED FROM PATIENTS AFFECTED BY AUTOIMMUNE DISEASE
Dipartimento Scienze Cliniche e Molecolari, Università Politecnica delle Marche, Ancona, Italy

INTRODUCTION. Scleroderma (SSc) is a systemic autoimmune disease of unknown origin, characterised by microvascular injury, autoimmune inflammatory responses, and severe and often progressive fibrosis (1). SSc fibroblasts isolated from lesional areas of patients overproduce reactive oxygen species (ROS) and overexpress type I collagen and α-smooth muscle actin (α-SMA) (2). Our group identified in the serum of SSc patients the presence of stimulatory anti-PDGFR receptor (PDGFR) auto-antibodies (SSc IgG) that are able to induce ROS production and fibrosis in normal fibroblasts (3-5).

There is evidence that SSc patients are characterised by activation of humoral immunity and B lymphocytes play a role in the pathogenesis of scleroderma (6). Rituximab is a monoclonal antibody which selectively targets and depletes CD20+ B lymphocytes, possibly influencing the serum levels of autoantibodies. In this work we investigated the biological effects of Rituximab in
patients affected by scleroderma with severe skin involvement.

METHODS. Six patients with severe skin fibrosis, unresponsive to immunosuppressive treatment, were treated with 375 mg/m\(^2\) per week of intravenous Rituximab for four doses. Stimulatory anti-PDGFR autoantibodies were detected. Fibroblast activation was evaluated in fibroblasts isolated from skin biopsies performed at baseline and at months 3 and 6 post-treatment. The modified Rodnan’s skin score, health assessment questionnaire (HAQ) and visual analogic scale (VAS) for global wellness and B lymphocyte count was performed monthly.

RESULTS. A significant reduction of anti-PDGFR autoantibodies was observed in the serum of all patients after 3 months of treatment. SSc fibroblasts showed a significant downregulation of type I collagen gene expression and intracellular signalling inhibition triggered by anti-PDGFR autoantibodies. A decrease of the skin score and an improvement of disability indexes matched with the in vitro results.

CONCLUSIONS. These data provide further evidence of B-cell involvement in the pathogenesis of scleroderma. Targeting B cells may be a promising treatment for scleroderma patients, and controlled clinical trials are warranted.

**BEST POSTER PRESENTATION**

Shkumbin A Thaqi

**ASSESSMENT OF KNOWLEDGE ABOUT THE BARC CENTRE AND SATISFACTION WITH THE EDUCATIONAL SERVICES AVAILABLE**

Rheumatology Resident
Republic of Kosovo

The aim if this proposal is to determine knowledge in the general population about arthritis and Immunodeficiency Syndrome; 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. It is sited in the city centre public library and works alongside the regular medical NHS service provision. Services for Rheumatology have always had lower priority (and funding) than those for acute services such as Cancer and Heart disease. In addition they have historically been somewhat restricted in the West Midlands (the UK region where Birmingham is the central city) compared to the rest of the UK. The picture is also complicated by the high percentage of ethnic minority groups locally (generally referred to as BME groups – Black and Minority Ethnic). Birmingham is set to become the first major UK city where BME groups will become the majority within the next ten years. There is evidence that “excluded groups” - such as immigrants, the poor and the less-well educated – have poorer health but do not access the NHS in the same way as the white middle-class population for a variety of reasons, including cultural, language and poverty
barriers. This is clearly relevant to the wider European scene where there is increasing pressure from immigration while currently both health and social programmes are threatened by the financial recession.

The BARC project was started a decade ago with a formal research process to determine the extent of current services and what people wanted. This “Needs Assessment” showed that both medical profession and public perceived a need for more information provided in an informal setting (ie a non-medical setting) - and wanted it in a range of languages (Adab et al Rheumatology 2004). There was also a widespread desire for more support services for patients. The BARC centre was set up on the basis of this in space provided by the City in the Central Library and is manned by volunteers. These have been selected and trained by the Centre manager, Chan Gordhan, who has a long background in social and voluntary work. The volunteers come from a range of ethnic backgrounds and importantly they have all had some personal rheumatic problem. Thus they fit what the UK government is now calling “expert patients” - and promoting the idea that they are best placed to help others since they have learnt how to cope. Interestingly our experience shows that volunteering to help others also empowers them to deal with their own lives, so they should also be the best placed group to teach us how to empower our clients. Our data also shows that the BARC service is wanted as well as needed locally.

The key point in developing any new service is to provide an evidence base for it. BARC set out to do this from the outset. Following the initial "Needs Assessment" We carried out a focus group study to determine what patients from BME groups were looking for from the local health services (Bacon’05). A key factor expressed by the participants was the desire to be listened too. They were dissatisfied with their doctors who were seen to lack time to take in the patients broader complaints. This echoes wider concerns about poor doctor – patient communications – an area which the Royal College of Physicians is holding an enquiry into at present.

BARC has set up sympathetic listening as one of the basic parts of the service (Gordhan’03 & ‘08). This is provided by trained volunteers. They are themselves patients and come from a range of ethnic and linguistic backgrounds, so that they are able to provide culturally sensitive guidance to clients. We have collected data on who has attended and how satisfied they are with the service provided (Treharne’04). Approximately 40% of attendees come from the BME groups, similar to the general population. Thus we are getting through to target populations - but not in large enough numbers. We have also had high gradings for client satisfaction.

We have also addressed the need for relevant patient-education material understandable to those for whom English is not their mother tongue. We recently completed a set of educational leaflets, designed as "bottom-up" material - that is based on questions people actually ask rather than information doctors think patients ought to know. They are in simple English, avoiding technical terms, so as to be easily understood. The first six have been translated into Urdu and recorded on CD's in both languages, as well as in print format with a few cartoons to illustrate them. A preliminary piece of market research in the BARC Centre shows that the volunteers think they are what is needed (and a small sample of clients listening to the first one agreed). The Urdu translation has also been approved by a range of Indian colleagues as being both true to the English information and understandable by a range of local language speakers. The translation is not strict Urdu but includes phrases used in Bollywood films (watched by all the local S.Asian groups) as well as some English words generally used in the version of “Urdu” widely used around Birmingham.

A questionnaire-based assessment of the first of these CD’s – on Understanding Arthritis – showed that clients gave it high scores for clarity of information and obtaining information that they
wanted. In general they found the CD helped them to cope (Sharif’08). We are just completing an assessment of the CD on rheumatoid arthritis and the outcome is very exciting. The challenge was far greater here as the usefulness of the CD was examined in a specialist RA clinic which already had a highly trained specialist nurse providing explanations and support to patients. Despite this the comments made at the focus groups demonstrated that the study participants had found the additional BARC service a major help (Kumar et al’09). There is now patient pressure to set up such a service on a regular basis in the hospital setting. This would be in line with the recent Report from the influential Kings Fund which noted a lack of understanding on the quality of RA care and the struggle many RA patients have to access quality care (Kings Fund’09).

In the same way, we have struggled to reach our target for new attendees at the BARC centre, despite the evidence for the need for and the success of the BARC service. Total numbers accessing the BARC service, including phone calls and web-site hits, have increased year-on year but surprisingly there has been no increase in personal visitors. A number of community centres have asked for the manager to go out to specific groups with promotional and educational talks. This alternative approach has proved very popular but many attendees have said they were not aware of the BARC centre. These outreach sessions are demanding on Chan Gordhan’s time and there is an excellent service available at the library. Thus the next essential step is a study of why people are not coming in the predicted numbers.

**Hypothesis**

We propose that the population in general tend to downplay the importance of their musculoskeletal problems. This is reinforced by the poor publicity that arthritic diseases get compared to some others. Analysis of the relative importance given by press or TV showed that heart disease and cancer got far more attention and were treated as serious scientific problems. Rheumatic diseases by contrast were seen as “lifestyle problems” for which there was no real medical treatment. The existence of a ground-breaking local service does not appear to have changed that mould to any major extent. Each time that the BARC Centre has been discussed on local radio there has been a sharp rise in client enquiries – but only for a short period. We intend to analyse the degree of local awareness of the BARC and at the same time look further into the responses of those who do actually come to seek help.

**Methods**

The first aspect will be carried out by collecting data about knowledge of BARC and satisfaction with current educational support using standardised questionnaires. This will target both a random population (people accessing the Central Library for any purpose) and specific communities such as local Sikh and Somali populations who have already identified a perceived need for an increased service for their groups. A minimum of 200 library people will be sampled at random in each grouping. The second part (analysis of satisfaction with current services) will be completed by analysis of the data collected over the past two years from attendees at the Centre, who are all asked to complete such a form. The data from this project will be compared to that obtained 10 years ago in the original Needs Assessment.

**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. The latter has many messages for someone practicing in a major city with an immigrant population and you have already reported working with several ethnic minority groups in Kosova.. We have been thinking about this project for some time, so there are some things already in place to facilitate your research. We have already trialled a simple questionnaire for these assessments. A
sociology student is currently using these to collect some preliminary data from library visitors. That experience will focus the further development of the project. A trained health psychologist is available to help with analysing the questionnaires and the unstructured material coming from the “free comment” section at the end of each form. In the same way, the set of forms collected from clients attending the Centre in its early years have been analysed and will form a useful comparison with the planned analysis of the comments collected from recent clients. This exercise will definitely lead to at least one published paper. The methodologies used will be of value to you in assessing the worth of conventional treatment options across the field of rheumatology. Our speciality deals with incurable chronic disease and there is increasing evidence that patients have a different perspective on the outcome to their doctors (Hewlett’03). Helping people to cope with chronic disability, improving their life by addressing their real concerns rather than measuring “medical outcomes” like degree of swelling or ESR, is becoming increasingly important. Finding ways of reaching out to the large percentage of the population who have a disability related to a rheumatic problem is also essential to persuade politicians to take the subject seriously (and invest in it!). Thus the experience gained from this would be advantageous to your career in many ways — and I believe you would find working in BARC both interesting and rewarding. Once in place here you can join in all the usual University Rheumatology Departmental activities, from seminars to clinical meetings. We would also work to get you some exposure to Rheumatoid Arthritis clinics as an observer on an informal basis. That will be easier to do with colleagues on the ground than to set up formally in advance with the current NHS bureaucracy.

You will have free time to catch up on your reading, particularly on the fairly large literature on self-coping and on what people expect from health services. You would need this to write a good paper and I would expect you to write up a comprehensive introduction and methods section well before data collection has been completed. Of course we will be available to discuss that with you — but it will be your responsibility to produce the first version. I believe that an important part of such a fellowship is learning how to plan and write up your own research projects for the future.

**BEST POSTER PRESENTATION**

Dan Yang

**ADA2 DEFICIENCY ATTENUATES M2 MACROPHAGES DIFFERENTIATION**

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Loss-of-function mutations in the *CECR1* gene (Deficiency of adenosine deaminase 2 (ADA2); DADA2) have not only been linked to early-onset stroke, vasculopathy, inflammation, but also bone marrow failure. To further study the disease mechanism, we established a reliable patient-specific *in vitro* disease cell model by generating hiPSC-derived monocytes (iMono) and differentiated them to different macrophage subtypes (M0, M1 and M2) by M-CSF method. Results: Consistently with the primary cells, hiPSC-derived DADA2 cells expressed lower levels of M2 macrophage markers, indicating that the ADA2 deficiency leads to disrupted M-CSF signaling. This was further confirmed in one patient with homozygous mutation leading to a premature stop codon, c.794C>G, p.Gln265Stop, in *CECR1* encoding ADA2. Cytokine array data showed levels of
multiple M2 cytokines, including CCL22, CCL17, CCL20, and CXCL13, were lower in ADA2 deficient M0 and M2 macrophages. To determine the enzyme function of ADA2, we measured adenosine accumulation by LC/MS and found an impaired adenosine deaminase function with subsequent more accumulation of adenosine in ADA2-deficient U937 cells and hiPSC-derived ADA2 deficient M2 macrophages. In human primary macrophages, addition of adenosine leads to downregulation of M2 markers, while recombinant human ADA2 partially rescued this phenotype. Thus, the adenosine-mediated pathways may partially contribute to macrophage defects in DADA2. To further study the mechanism, we performed RNA sequencing analysis in U937 cells. Lack of ADA2 resulted in downregulation of genes associated with PMA-induced adhesion, differentiation, chemotaxis, and inflammation. Pathway assay suggests IL10 is playing a central role in ADA2-mediated cell maturation and differentiation. By immunoblotting, we show deceased PMA-induced PKC-delta and P38 activation in ADA2 knockdown U937 cells. The role of the ADA2/PKC/P38 pathway in patient-specific cells needs to be determined. Conclusion: Deficiency of ADA2 attenuates M2 macrophage differentiation. Together current data demonstrate a role for adenosine and PKC/P38 signaling pathways in the ADA2-mediated macrophage differentiation. The altered ratio of tissue M2/M1 macrophages likely contributes to inflammation and the vasculopathic/vasculitis phenotype in patients with DADA2.

**POSTER PRESENTATION**

**Marina Zafranskaya**

**IMMUNOLOGICAL MONITORING OF MULTIPLE SCLEROSIS PATIENTS AFTER AUTOLOGOUS MESENCHYMAL/STROMAL STEM CELLS THERAPY**

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**Background:** The absence of protocols for multiple sclerosis (MS) effective treatment aimed at autoreactive T-lymphocytes selective suppression explains the increased research interest to immunomodulatory and neuroprotective properties of mesenchymal/stromal stem cells (MSC). **Patients and design.** Patients with relapse-remitting MS (n=12, f:m – 6:6), mean age – 33,0(25,5÷35,5) years, with expanded disability status scale score (EDSS) of 2,75(2,00÷3,25) received autologous bone-marrow-derived MSC (1,55(0,92÷2,35) cells/kg) intravenously. 12 months follow-up included neurological investigation and immunological tests. **Results.** Within one year after therapy, the negative clinical-neurological dynamics was registered in 3 (25,0%), improvement in 2 (16,7%) and stabilization in 7 (58,3%) patients. The predictive value of in vitro model of lymphocytes and MSC cocultivation and immunomolecular markers have been determined for assessing the in vivo immunomodulatory effect of cell therapy in MS patients using the index of MSC suppression of myelin-induced T cells proliferation. The intravenous administration of autologous MSC to MS patients resulted in increased number of γδT-lymphocytes, reduced number of circulating memory CD45RO positive T cells and suppressed myelin-stimulated T cells proliferation within 12 months after transplantation that characterize in vivo immunomodulatory effects of cell therapy. In MS patients after autologous MSC transplantation, there was no marked inhibition of T-lymphocytes non-specific stimulation that is
evidenced by low mitogen-induced proliferation suppression indices within one year after cell therapy.

**Conclusion.** The dynamics of T-lymphocytes functional state to myelin-specific antigen, number of γδT-lymphocytes in peripheral blood and CD45RO expression on T cells after cell therapy make it possible to establish the correlation between the *in vitro* and *in vivo* effects of MSC.

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**TOPIC:** CANCER AND VIRAL IMMUNITY

**BEST POSTER PRESENTATION**

Banu Bayyurt

ENCAPSULATION OF STING AND TLR9 LIGANDS IN PH-SENSITIVE LIPOSOMES RESULTS IN POTENT ANTICANCER IMMUNITY AND REDUCES ESTABLISHED TUMORS

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STING ligands (cyclic dinucleotides, CDNs) are potent type-I interferon (i.e. IFN-α/β) inducers. However, *in vivo* potential of CDNs is hampered due to their limited uptake by STINGexpressing cells. Previously, we demonstrated that a robust Th1-biased and pronounced IFN-α/β response is generated upon co-injecting CDNs and CpG-ODN [1]. In this study, we aimed to increase cellular uptake of CDNs and TLR9 ligands and enhance their *in vivo* immunostimulatory potential by co-encapsulating them within liposomes. For that, we used pH-sensitive modified sterically stabilized cationic liposomes (SSCL hereafter) [2,3]. The pH-sensitive SSCL would leak CDNs into cytosol allowing STING sensing and keep CpG-ODN in close proximity with TLR9 within late endosomes. Preliminary studies were focused on the determination of the pHsensitivity of the generated liposomes. We altered the pH-sensitive lipid (i.e. dioleoylphosphatidylethanolamine-DOPE) ratio. Data revealed that calcine release at pH:4.0 was maximum when DOPE ratio was raised from 5 to 7. Then, we co-encapsulated CpG-ODN and CDNs within liposomes via lyophilization technique [4]. Splenocytes, bone marrow-derived dendritic cells (BM-DC s) and macrophages (BM-DMs) were stimulated with free or liposomeloaded ligands (0.1 μM to 3.0 μM concentration range) for 24h. IL-6, IL-12, IFN-γ and IFN-α/β productions from immune cells were determined. Data implicated splenocytes that were stimulated with liposome co-encapsulating dual ligands boosted IFN-γ and IFN-α/β levels (≈60- and ≈6-fold, respectively, p<0.001). More importantly, BM-DCs and BM-DMs after stimulation with liposome-loaded dual ligands led to significantly higher IL-12 secretion (≈7-fold induction, p<0.001). Of important note, when tested on human PBMCs liposomal formulation replicated their potent stimulatory effects. There was >18-fold more IFN-α secretion in hPBMCs stimulated with liposome-loaded dual ligands than free or liposome-loaded single ligands (p<0.001). *In vivo* efficacy of the liposomal formulations was tested on the B16-F10-OVA tumor bearing mice. One week after tumor engraftment, C57BL/6 mice developed palpable tumors (≈100 mm3). Mice were treated on alternating days for a week either with free or liposome-loaded dual ligands (1.7 μg CDN and 5 μg CpG-ODN each injection). Animals were followed for 10 days and
tumor volumes were recorded. Results showed that dual ligands loaded liposome therapy led to 80% remission of tumors. Next, OVA-specific total IgG, IgG1 and IgG2c titers were determined from sera. We detected significantly elevated IgG2c/IgG1 ratio in animals treated with liposomal formulations, indicating the development of antigen specific Th1 biased immunity. Furthermore, liposome co-encapsulating CpG-ODN and CDN significantly elevated antigen dependent IFN-γ producing CD8+ T-cells and significantly decreased M2-type macrophages at the tumor bed. In conclusion, co-encapsulating CDNs and ODNs into pH-sensitive liposomes elicited tumor specific CD8+ T-cells and lead to significant reduction of the established melanoma tumors.

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**BEST POSTER PRESENTATION**

Dr. Bérengère Salomé

ABSTRACT FOR THE SESSION “CANCER AND IMMUNITY” KILLER ILCs (ILCK): A NOVEL METABOLICALLY DISTINCT, CYTOTOXIC ILC SUBSET PREDICTIVE OF AML PROGNOSIS

Ludwig Cancer Center Research of the University of Lausanne - Department of Fundamental Oncology, University Hospital (CHUV), Lausanne, Switzerland

Innate Lymphoid Cells (ILCs) are a recently identified family of lymphocytes constituted of at least 3 different groups that mirror the functional specialization of CD4 T cells. ILCs have been shown to play important roles in inflammatory diseases and cancers, mainly in mice models. In contrast, the role of these cells in humans remains poorly characterized.

We performed detailed phenotypic and functional studies of Lin-CD127+ innate lymphoid cells present in both the peripheral blood from 47 healthy humans and in lymphoid and non-lymphoid healthy human tissues. We identified a new subset of ILC, hereafter named “Killer ILCs” (“ILCK”). These ILCs have an expression pattern of chemokine-, interleukin-, natural killer cell-related and death-receptors distinct from other ILCs and conventional natural killer (cNK) cells. Using mRNA sequencing of highly pure ex-vivo flow-cytometry based sorted ILCs and cNK subsets, we show that ILCK, ILC3 and CD16negCD56bright NK cells form a cluster based on their transcriptomic profile. Interestingly, we identified a specific ILCK gene signature, which distinguishes these cells from the other human ILC and cNK subsets. We used functional assays to demonstrate that ILCK produce granzymes and perforin and are capable of killing tumor cell lines. Metabolic profiling shows that ILCK display robust mitochondrial activity compared to other ILC and cNK subsets, highlighting the potential advantage of these cells for survival in glucose-depleted tumor micro-environment. Moreover, we observed that ILCK frequencies at diagnosis correlate with tumor progression and clinical outcome of acute myeloid leukemia patients.

Overall, we identified a new cytotoxic ILC subset with putative key roles in tumor recognition, thus representing a potential prognosis tool and target for tumor immunotherapy.

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**BEST POSTER PRESENTATION**

Francesca Calcaterra
ENDOTHELIAL COLONY-FORMING CELLS ISOLATED FROM CLASSIC KAPOSÍ’S SARCOMA PATIENTS ARE ENDOWED WITH CELLULAR FEATURES THAT EVOKE KS SPINDLE CELL BEHAVIOR

Department of Medical Biotechnologies and Translational Medicine, University of Milan, Italy. Unit of Clinical and Experimental Immunology, Humanitas Clinical and Research Center, Rozzano, Milan, Italy.

Background: Kaposi’s sarcoma (KS) is a lymphoangioproliferative tumor characterized by hyperproliferation of spindle cells, the typical component of KS lesions. Spindle cells are cells of endothelial origin that assume their characteristic features upon infection with human herpesvirus-8 (HHV8), the causative agent for KS. The multifocal nature of KS suggests that spindle cells derive from circulating HHV8-infected precursors that yet lack identification. Endothelial progenitor cells (EPCs) are rare bone-marrow derived cells highly involved in vasculogenesis and angiogenesis and, among population of putative EPCs isolated and cultured from adult blood, endothelial colony-forming cells (ECFCs) are considered the true EPCs as they are unique in their ability to sustain adult blood vasculogenesis. In a previous study we demonstrated that ECFCs obtained from classic KS (cKS) patients are HHV8-infected, suggesting that these cells may be putative precursors of KS spindle cells. In order to underpin this hypothesis, in the present study we investigated whether ECFCs isolated from cKS patients have features and in vitro behavior similar to the spindle cells in KS lesions.

Methods: ECFCs were obtained from 83 cKS patients and 86 healthy HHV-8 seronegative controls, using a culture protocol optimized in our lab. During the isolation phase, PBMCs were seeded in EGM-2 medium in culture plates coated with fibronectin and ECFC colonies were identified as cluster of adherent cells with cobblestone-like morphology. The time of appearance and the frequency of ECFC colonies were analyzed in order to evaluate the efficiency of ECFC colony isolation. Once isolated, ECFC colonies were cultured in plates coated with collagen. During the expansion phase, ECFC phenotype and the presence of HHV8-infection - assessed as expression of the viral latent nuclear antigen (LANA) - were analyzed by immunofluorescence. Moreover, ECFC were functionally characterized by evaluating their cell viability, proliferative potential, vasculogenesis ability by Matrigel assay and cytokine production by ELISA.

Results: During the isolation phase, ECFC colonies appeared earlier (p<0.0001) and with higher frequency (p<0.0001) when isolated from cKS patients compared with healthy controls. During the expansion phase, ECFCs obtained from both cKS patients and controls were endowed with the typical characteristics of ECFCs. However, ECFCs isolated from cKS patients were endowed with a significantly higher proliferative and vasculogenic potential (p<0.05) and produced higher levels of IL-6 than control ECFCs (p<0.05) and were characterized by an activated immunophenotype as they expressed at higher levels ICAM-1, VCAM-1 and E selectin (p<0.05). Finally, only ECFCs isolated from KS patients resulted HHV8-infected. In particular, similarly to spindle cells in KS lesions, only a variable proportion of cells within ECFC colonies isolated from cKS patients were HHV-8 infected as confirmed by the positive staining for LANA.

Conclusions: These results demonstrate that ECFCs obtained from KS patients have features that evoke the KS spindle cell behavior, thus reinforcing the hypothesis that ECFCs may be bone marrow-derived HHV8-infected precursors, that upon localization in peripheral tissues may differentiate into typical spindle cells, and undergo intense proliferation and angiogenesis under the effects of cofactors present in the local microenvironment.
BEST ORAL PRESENTATION

Francesca Conti

EPSTEIN-BARR VIRUS CHRONIC INFECTION IN TWO PATIENTS WITH APDS SYNDROME: A COMBINED RISK FACTOR FOR DEVELOPING LYMPHOPROLIFERATIVE DISORDERS.

University Department of Pediatrics, Unit of Immune and Infectious Diseases, Childrens' Hospital Bambino Gesù, Rome, Italy

Background: Activated PI3K Delta Syndrome (APDS) is a primary immunodeficiency disease caused by activating mutations in either the leukocyte-restricted p110δ catalytic (PIK3CD) subunit or the p85α regulatory (PIK3R1) subunit, predisposing to lymphoma. Moreover, PI3K/AKT/mTOR pathway is known to be inappropriately activated in various cancer due to somatic activating mutations and during Epstein-Barr virus (EBV) infection, leading to increased risk to develop lympho-proliferative disorders (LPDs).

Objective: We report our experience in diagnosis and management of two APDS patients presenting with EBV chronic replication and LPDs.

Results: The first case is a 30-year-old girl. The patient presented since early life, with recurrent sinopulmonary infections, lymphadenopathy and failure to thrive. Chronic EBV infection was detected during infancy. At 10-year-old immunological investigations showed CD4+ naive lymphopenia and dysgammaglobulinaemia consistent with a Hyper-IgM syndrome; no mutations were identified however monthly immunoglobulin infusion was started. At 18-year-old diagnosis of Hodgkin's lymphoma successfully treated with chemotherapy and radiation therapy complicated by ovarian torsion and early menopause. At 24-year-old, she presented abdominal pain and bloody diarrhoea. Endoscopic evaluations showed colonic micro erosions and multiple sessile polyps. Histological finding was suggestive of IBD-like picture. She started treatment with mesalazine with annual endoscopic follow up. Mutation in PIK3R1 (c.1425+1G>T) was identified at the age of 30 after revaluation of the clinical and immunological picture. The patient was asymptomatic and during a routinely colonoscopy was identified a polypoid mass with histological finding of diffuse large B-cell lymphoma. The patient was referred to adult center to perform chemotherapy. The second patient is a 19-year-old boy, followed for a combined immunodeficiency with Hyper-IgM since 5 years of age. Mutation in PIK3CD (c.3061G>A) was found at 16-year-old. He presented multiple lymphadenopathies requiring whole biopsy, characterized by hyperplasia involving cortical and the germinal center. EBV chronic replication infection was observed since childhood. The patient remained in good clinical conditions with monthly immunoglobulin infusion. At 19-year-old, he started to complain fatigue, increase of lymph node size, weight loss and diarrhea. We performed an additional lymph nodes biopsy and of duodenal mucosa through a digestive endoscopy that were diagnostic for an extra nodal large cell lymphoma B, EBER positive. He was successfully treated with chemotherapy.

Conclusion: The activation of PI3Kδ signaling contributes to B cell proliferation with a higher risk of neoplastic transformation. Indeed, APDS patients have a high incidence of lymphoma, since young adulthood. Importantly, gastrointestinal LPDs localization may be silent, so clinicians should consider early and alternative diagnostic tools as scheduled laboratory and imaging exams including biopsy when LPDs are detected, upper and low endoscopies and involvement of a
A multidisciplinary team including radiologist, hematologist and gastroenterologist with strict follow up even without specific symptoms. Inhibiting the PI3K/AKT pathway or its downstream proteins using drugs already approved for other diseases may reduce malignancies associated with latent herpesvirus infections. Deep studies of these PIDs and their susceptibility to EBV are needed to understand the affected molecular pathways in order to modulate immune response to control the EBV infection and LPDs.

**BEST POSTER PRESENTATION**

Filippo Cortesi

**iNKT cells control prostate cancer progression by differentially modulating tumor associated macrophages**

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CD1d-restricted invariant (i)NKT cells are T lymphocytes displaying innate effector functions strongly implicated in tumor immunesurveillance. Transgenic oncogene-induced prostate cancer (PC) in mice lacking Inkt cells is more precocious and aggressive, leading to reduced survival compared to iNKT cell-sufficient animals. We investigated the mechanisms subtending the iNKT cell control of mouse PC. Compared to WT mice, tumors of iNKT cell-deficient hosts exhibit an extensively modified tumor microenvironment, containing increased pro-angiogenic Tie2+ tumor associated macrophages, enlarged vascular areas and decreased proinflammatory CD11c+ tumor associated macrophages. iNKT cells are directly involved in the modulation of tumor associated macrophages and control of tumor progression, which critically depends on CD1d recognition and entails: i selective support to pro-inflammatory macrophage survival via CD40L-CD40 engagement; ii killing of pro-tumor ones via FasL-Fas; and iii promotion of macrophage differentiation toward pro-inflammatory phenotype. Lack of Inkt cells upregulates a pro-angiogenic transcriptional signature in mouse PC that is conserved in more aggressive human tumors. Hence, iNKT cells can restrain PC progression by non-redundant mechanisms that enforce a tumoropposing microenvironment via differential modulation of TAM populations.

**BEST ORAL PRESENTATION**

 Daniela Angela Covino

**WHOLE TRANSCRIPTOME PROFILING OF PRIMARY HUMAN MACROPHAGES IDENTIFIES AN ASSOCIATION BETWEEN CCL2 NEUTRALIZATION-MEDIATED INHIBITION OF HIV REPLICATION AND DEREGULATION OF HOST GENES AND PATHWAYS INVOLVED IN ANTIVIRAL RESPONSES**

National Center for Global Health, Istituto Superiore di Sanità, Rome, Italy

Macrophages are key targets of HIV infection and a major source of CCL2, a proinflammatory chemokine highly expressed in HIV infected subjects. CCL2 expression is enhanced during monocyte differentiation to macrophages and further increased in HIV infected cells, where it
promotes viral replication. Recently, we showed that CCL2 neutralization profoundly restricts HIV-1 replication in macrophages, affecting post-entry steps of the viral life cycle independently of SAMHD1. To define the cellular correlates of protection, we performed transcriptome profiling to assess the effect of CCL2 neutralization on global gene expression. Total RNA, extracted from macrophages exposed to anti-CCL2 or control antibody for 4 and 20 h, was subjected to poly(A) selection, reverse transcription, generation of cDNA libraries and sequencing on an Illumina HiSeq 2500 platform. Differential expression analysis was done using DESeq2. Genes with $\log_{2}F_{C}\geq1$ (upregulated) or $\log_{2}F_{C}\leq-1$ (downregulated) and adjusted p-value $<0.1$ were classified as significantly differentially expressed. CCL2 blocking resulted in the differential expression of 1915 and 311 genes at 4 and 20 h, respectively. The majority of these genes were specifically modulated by anti-CCL2 antibody, whereas only a minor fraction of genes were modulated also by control antibody. In order to gain further insight into the specific categories of genes whose expression is changed upon treatment with anti-CCL2 antibody, we performed annotation and pathway analysis using DAVID and GOrilla. This analysis revealed that “immune system process”, “defense response”, “immune response”, and “innate immune response” were among the top ten GO biological terms with the higher number of upmodulated enriched genes. Interestingly, among these genes we found the host restriction factors Mx2 and APOBEC3A (A3A), whose mechanisms of action in viral inhibition may account for the post-entry restriction of HIV-1 replication mediated by CCL2 blocking in MDMs. We thus performed qPCR and western blot experiments to confirm the differential expression profiles of these factors at either the transcriptional or protein level. Although the upregulation of both genes following CCL2 neutralization was confirmed by qPCR, an increase of protein level was observed only for A3A. Interestingly, A3A protein levels induced by CCL2 blocking were comparable to those of freshly isolated monocytes, where high levels of this factor were associated with resistance to HIV-1 infection. Overall, these data demonstrate that genes involved in innate antiviral responses are differentially regulated by CCL2 neutralization, suggesting that multiple mechanisms may contribute to HIV-1 replication restriction by CCL2 blocking. Since macrophages are potential viral reservoirs which produce replication-competent HIV virions even in the presence of effective combination antiretroviral therapy, targeting CCL2 may represent a new therapeutic strategy to strengthen innate intracellular pathways and protect macrophages from infection. Italian Ministry of Health, RF-2011-02347224.

POSTER PRESENTATION

Gloria Delfanti
INVESTIGATING AND HARNESSING INVARIANT NATURAL KILLER T LYMPHOCYTE INTERACTIONS WITH CANCER CELLS AND TUMOR-SUPPORTING STROMA
Experimental Immunology Unit, Division of Immunology; Transplantation and Infectious Diseases, San Raffaele Scientific Institute, Milan - Italy

Background: CD1d-restricted invariant (i)NKT cells are a subset of T lymphocyte displaying innate effector functions that is strongly implicated in tumor immune surveillance via direct and indirect mechanisms. The absence of iNKT cells in authochthonous or transplantable prostate cancer mouse models results in more aggressive tumors and decreased survival compared to iNKT cell-sufficient
mice. iNKT cells modulate a population of pro-angiogenic tumor associated macrophages (TEMs) via CD1d recognition and CD40-CD40L and Fas-FasL engagement. We now aim at enhancing iNKT cell immunotherapy by engineering them with TCRs specific for MHC-restricted tumor-associate antigens (TAAs), in order to achieve dual targeting of TEMs and cancer cells. We hypothesize that the concurrent targeting of both tumor and stroma by Inkt cells should enhance their clinical efficacy upon adoptive immunotherapy.

Materials & Methods: iNKT cells are expanded in vitro and transduced with retroviral vectors encoding pmel, OT1, or TagIV TCRs, specific for gp100, Ova, SV40LT tumor antigens, respectively. The activity of transduced iNKT cells is evaluated by assessing the recognition in vitro, and the control in vivo, of different tumor cells expressing these Ags.

Results: The exogenous TCRs can be efficiently transduced into mouse iNKT cells, where they are variably co-expressed with the endogenous TCR. The reactivity of the engineered iNKT cells is currently being assessed.

Discussion and conclusion: Our preliminary results support the feasibility of the TCR transfer strategy into iNKT cells.

BEST ORAL PRESENTATION

Maria Rosaria Galdiero
NEUTROPHIL PLASTICITY IN THYROID CANCER

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Background: Neutrophil function has long been viewed limited to the acute phase of inflammation and resistance against pathogens. The role(s) of neutrophil in tumor initiation and progression remain poorly understood. Neutrophils are among the inflammatory cells infiltrating the tumors and recent studies placed them as key effector cells in the orchestration of the immune and inflammatory responses. However, the association between neutrophil infiltration, clinicopathological features and outcome in cancer patients remain to be clarified. Thyroid cancer (TC) is the most frequent type of cancer of the endocrine system, accounting for 70% of deaths due to endocrine cancers. No studies are so far available investigating the role of neutrophils in TC.

Objective: The objective of this study was to investigate the role of tumor-infiltrating neutrophils in TC.

Methods: Highly purified human neutrophils (>99%) from healthy donors were stimulated, in vitro, with conditioned media derived from the thyroid cancer cell lines TPC1 and 8505c (TC-CM). Neutrophil functions (e.g. chemotaxis, activation, plasticity, survival, gene expression and protein release) were evaluated.

Results: We found that TC cell lines produced soluble factors able to promote neutrophil chemotaxis and survival. In particular, neutrophil chemotaxis toward TC-CM was mediated, at least in part, by CXCL1/Gro-α and CXCL8/IL-8. Neutrophil survival induced by TC-CM was mediated by GM-CSF. In addition, TCCM induced neutrophil morphological changes and activation (CD11b and CD66b up-regulation, CD62L shedding) and modified neutrophil kinetic properties. Furthermore, TC CM induced the production of reactive oxygen species (ROS), the expression of pro-inflammatory and angiogenic stimuli (CXCL8/IL-8, VEGF-A) and the release of matrix metalloproteinase-9 (MMP-9). Preliminary experiments indicate that “tumor-educated
neutrophils” co-cultured with TC cells favor tumor cell proliferation in vitro.
Conclusions: TC cell lines produce soluble factors able to ‘educate’ neutrophils towards an activated functional state. Experiments are in progress to better understand the role of these “tumor-educated neutrophils” in modifying TC behavior. These data will advance our understanding on the molecular and cellular mechanism of the innate immune system in thyroid cancer.

POSTER PRESENTATION
Griffante Gloria

THE HUMAN CYTOMEGALOVIRUS TEGUMENT PROTEIN PP65 (PUL83) DAMPENS INTERFERON-TYPE I PRODUCTION BY INACTIVATING THE DNA SENSOR CGAS
Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy

The innate immune response against human Cytomegalovirus (HCMV) plays a pivotal role during primary infection. Indeed, HCMV infection of primary fibroblasts rapidly triggers strong induction of type I interferons (IFNs) accompanied by proinflammatory cytokine release. Here, we show that primary human foreskin fibroblasts (HFFs) infected with TB40/E unable to express UL83-encoded pp65 (v65Stop) produce significantly higher type I IFN levels than HFFs infected with the wild type HCMV strain TB40/E (v65Rev), suggesting that the tegument pp65 protein may dampen type I IFN production. To clarify the mechanisms through which pp65 inhibited type I IFN production, we analyzed the activation of the cGMP-AMP synthase (cGAS)/STING/IRF3 axis in HFFs infected with either v65Rev or pp65-deficient mutant v65Stop. We found that pp65 selectively binds to cGAS and prevents its interaction with STING, thus inactivating the signaling pathway through the cGAS/STING/IRF3 axis. Notably, within the first 6 hours of HCMV infection, STING undergoes proteasome degradation independent of the presence or absence of pp65. Additionally, downregulation of cGAS activity by pp65 blocked IRF3 dimerization and DNA binding activity, leading to the inhibition of type I IFN production. Collectively, our data provide mechanistic insight into the interplay between HCMV pp65 and cGAS, leading to subsequent immune evasion by this prominent DNA virus.

BEST POSTER PRESENTATION
Ewelina Grywalska

EXPRESSION OF CD25 AND CD69 ON T AND B LYMPHOCYTES IN CHRONIC LYMPHOCYTIC LEUKEMIA AND ESTABLISHED PROGNOSTIC FACTORS OF THIS CONDITION
Department of Clinical Immunology and Immunotherapy, Medical University of Lublin, Lublin, Poland

The aim of this study was to determine the percentages and absolute numbers of lymphocytes B and T in peripheral blood and bone marrow of chronic lymphocyte leukemia (CLL) patients. Moreover, we analyzed relationship between the number of CD25- and CD69-positive lymphocytes and established prognostic factors in CLL. The study included 80 untreated patients
with CLL and 20 healthy subjects. The immunophenotype of peripheral blood mononuclear cells (in both groups) and bone marrow cells (solely in the CLL group) was determined by means of flow cytometry. Compared to healthy individuals, patients with CLL showed higher absolute number of activated lymphocytes B with phenotypes CD19+CD25+ and CD19+CD69+, as well as higher absolute number of CD3+CD25+ lymphocytes T. Enhanced activation of peripheral blood and bone marrow lymphocytes was associated with higher Rai stages, increased concentration of lactate dehydrogenase and beta-2 microglobulin and progression of the disease. Moreover, the number of lymphocytes B CD19+ZAP-70+ correlated positively with the number of CD19+CD25+ B cells and CD3+CD69+ T cells. Additionally, the relationship between the fraction of activated B and T lymphocytes and probability of survival after the diagnosis of CLL has been assessed. This study confirmed the association between negative prognosis and high expression of activation markers in CLL patients. Determination of CD25+ and CD69+ lymphocytes T and B constitutes valuable diagnostic tool, completing cytometric evaluation of CLL.

Keywords: cytometry, lymphocyte activation, prognosis, superficial antigens

POSTER PRESENTATION

Mohammad Azhar Kamal

EVALUATION OF NOVEL BIOMARKERS ASSOCIATED TO PANCREATIC CARCINOGENESIS FOR THE EARLY DIAGNOSIS OF PANCREATIC DUCTAL ADENOCARCINOMA

Dept of Immunology and Inflammation

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive forms of cancer and estimated to become a leading cause of cancer death by the year 2030. Early detection of this cancer is the key determining step to surgery eligibility of PDAC patients, in order to improve survival rate and prognosis. In this study, we report a differential quantitative proteomic approach for the global identification and quantification of secretome proteins in pancreatic KRasG12V-transformed cells to be compared with Mock-transduced cells. Proteins in cell conditioned media labeled with Stable Isotope Labeling with Amino acids in Cell culture (SILAC) were analyzed by 1DE-LC-mass spectrometry analysis with LTQ Orbitrap XL). Among the over-expressed proteins by KRasG12Vtransformed cells, were proteins linked to important biological pathways for tumor cells, which include: matrix-associated proteins, proteins related to KRas-signaling, metabolic enzymes and inducers of the epithelial-to-mesenchymal transition. A panel of 6 candidate proteins: LamininC2 (LAMC2), Tenascin-C (TNC), Stanniocalcin 2 (STC2), RAN-GTPase, Farnesyl Pyrophosphate synthase (FPPS), and Ubiquitin carboxyl terminal hydrolase-L1 (UCHL-1) was further selected to be validated in biological samples. Human PDAC patient’s tissue had high mRNA expression for these proteins. The circulating levels of LAMC2 and TNC, measured by ELISA, were significantly higher (p < 0.001) in PDAC patients (n=102) compared to healthy individuals (n=80). The ROC curve analysis (0.86 and 0.74, respectively) revealed good specificity and sensitivity. Furthermore, these candidate biomarkers were significantly higher already in early-stage patients, indicating potential for the early diagnosis of PDAC. This work is supported by AIRC 5X1000 project 12182.
Microenvironment or tumor derived signals activate innate immune cells and polarize them to a specific phenotype by a combination of epigenetic and transcriptional mechanisms. Microglia, brain specific innate immune cells, accumulate in gliomas and acquire the proinvasive and immunosuppressive phenotype supporting tumor progression. It is poorly understood how microglia are reprogrammed in a response to challenges and how signals are converted into sustained patterns of gene expression in brain inflammation. Transcriptome analysis of microglial cultures exposed to glioma (GCM) or lipopolysaccharide (LPS) shows activation of distinct signaling and metabolic pathways resulting in different patterns of gene expression. We assess DNA methylation and patterns of selected activating and repressive of histone modifications in tumor-related and inflammatory activation of microglia, to determine their roles in protumorigenic reprogramming. Epigenetic enzyme inhibitors were used to assess the role of histone modifications in a phenotype acquisition. We demonstrate unmethylated DNA and active histone marks at the promoter regions of inflammation, M1/M2 specific genes in unstimulated microglia. Increase of HDAC expression/activity specifically in glioma-activated microglia erases active histone marks. At later times genes regulated in stimulus-dependent manner acquire the repressive histone marks likely critical for phenotype consolidation. Microglia pre-exposed to glioma acquire an "epigenetic memory" and have considerably reduced inflammatory responses after inflammatory activation. HDAC inhibitors block glioma-driven transcriptional activation and restore an ability to launch effective inflammatory responses. Our study demonstrates how reversible epigenetic mechanisms may stably reprogram brain innate immune cells, which in consequence shapes antitumor responses in gliomas.

Key words: microglia, DNA methylation, histone modifying enzymes, histone deacetylases, inflammatory response, glioma-associated activation

BEST POSTER PRESENTATION

Lo Presti E.

DISTINCTIVE FEATURES OF TUMOR-INFILTRATING ΓΔ T LYMPHOCYTES IN HUMAN COLORECTAL CANCER

Central Laboratory of Advanced Diagnosis and Biomedical Research (CLADIBIOR), University of Palermo, Department of Biopathology and Medical Biotechnologies (DIBIMED), University of Palermo

Tumors grow in a complex and intricated network of epithelial and mesenchymal cells, inflammatory and immune cells and the characterization of the immune contexture is a major prognostic factor for patients survival and represent a target for innovative cancer therapies. We have studied the frequency, phenotype and functions of γδ T cells infiltrating CRC and correlated levels of intratumoral γδ T cells with any of the established clinicopathologic features described.
for CRC. The majority of γδ T cells in both CRC and adjacent normal tissues expressed Vδ1, but intratumoral γδ T cells did not exhibit a distinct prevalence and distribution of Vδ1 and Vδ2 T cell subsets, compared to adjacent normal tissue. Most Vδ1 T cells in tumor tissues were of effector memory phenotype, whereas intratumoral Vδ2 T cells had a more heterogeneous phenotype and both Vδ1 and Vδ2 T cells in CRC and adjacent normal tissues preferentially produce IFN-γ, but very low IL-17. Moreover, IFN-γ production by both Vδ1 and Vδ2 T cells is significantly reduced in the tumor tissue and culture supernatants from cancer stem cells significantly inhibited in vitro proliferation and IFN-γ production by both γδ, CD4 and CD8 T cells lines, thus indicating that inhibitory molecules produced in the tumor microenvironment have a profound effect on several components of T cell responses. Transcriptome analysis of 585 CRC samples showed that numbers of CRC-infiltrating total and IL-17- or IFN-γ-making γδ T cells neither correlated with clinicopathologic features of CRC, nor predicted disease free survival, but patients without lymph node metastasis containing high number of tumor-infiltrating total γδ T cells had significantly longer 5-year disease free survival rate, suggesting that intratumoral γδ T cells may be efficacious in controlling CRC at very early stage.

BEST POSTER PRESENTATION
Matteo Massara

HEMATOPOIETIC EXPRESSION OF THE ATYPICAL CHEMOKINE RECEPTOR 2 INHIBITS THE RECRUITMENT OF ANTI-METASTATIC NEUTROPHILS
Humanitas Clinical and Research Center, Rozzano, Italy; Department of Medical Biotechnologies and Translational Medicine, Università degli Studi di Milano, Rozzano

PURPOSE: The atypical chemokine receptor 2 (ACKR2) is a scavenger receptor for most inflammatory CC chemokines. It plays a protective role in chronic inflammation, autoimmunity and inflammation-related cancer. The aim of this project is to investigate the role of ACKR2 in primary tumor development and metastatization in different mouse model.

METHODS: NeuT mice (overexpressing the rat oncogene Her2) crossed with ACKR2/-/- mice, orthotopic and intravenous injection of the breast carcinoma cell line 4T1 in Wild Type (WT) and ACKR2/-/- Balb/c mice and intravenous injection of the melanoma cell line B16F10 in WT and ACKR2/-/- C57BL/6 mice. Tumors and metastasis were studied with in vivo imaging systems, immunohistochemistry and qPCR analysis; blood composition and metastatic organ infiltrate were studied with FACS analysis.

RESULTS: Tumor arising in ACKR2/-/- NeuT mice showed a more aggressive phenotype when compared to WT NeuT mice. On the contrary, ACKR2/-/- NeuT mice were protected from spontaneous lung metastasis. Metastasis protection was registered also using the 4T1 orthotopic model. FACS analysis indicated an increased of Ly6G+ polymorphonuclear cells and Ly6Chigh monocytes in the blood and in the pre-metastatic lungs of ACKR2/-/- mice. Depletion and adoptive transfer experiments demonstrated that neutrophils are implied in metastasis protection. Moreover, ACKR2/-/- neutrophils express increased levels of the chemokine receptor CCR2 and have increased CCR2 dependent cytotoxic activity.

DISCUSSION: ACKR2 is described to regulate leukocytes recruitment shaping chemokine in tissues but our evidences indicate ACKR2 as a direct regulator of immune cells activity.

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CONCLUSION: These results indicate that ACKR2 expression has a dual but opposite role in tumor, inhibiting primary tumor growth but promoting lung metastatization through the inhibition of anti-metastatic neutrophils.

BEST POSTER PRESENTATION

Meraviglia S

**ΓΔ T CELLS PRODUCING IL17 OR IFN-Γ ARE RECRUITED TO THE TUMOR SITE IN SQUAMOUS CELL CANCER AND DIFFERENTIALLY CORRELATE TO THE TUMOR STAGE.**

Central Laboratory of Advanced Diagnosis and Biomedical Research (CLADIBIOR), University of Palermo,
Department of Biopathology and Medical Biotechnologies (DIBIMED), University of Palermo

The identification of reciprocal interactions between tumor-infiltrating immune cells and microenvironment may help to understand mechanisms of tumor growth inhibition or progression. We have assessed the frequencies of tumor-infiltrating and circulating T cells and Treg cells from 47 patients with squamous cell carcinoma (SCC), in order to understand if they correlate with progression or survival. V1 T cells infiltrated SSC tissue at higher levels than found in normal skin and in PBMC of SCC patients and healthy subjects, while V2 T cells showed higher frequency in PBMC than in the tissue either in cancer patients than in healthy donors. Tumor-infiltrating T cells preferentially showed an effector memory phenotype and made both IL17 and IFN-γ, thus differing from circulating of SCC patients, which preferentially made IFN-γ. Moreover, the levels of IL17 producing T cells amongst tumor-infiltrating lymphocytes were significantly higher in SCC patients with advanced disease, while the levels of IFN-γ-producing T cells were higher in SCC patients at early stage of disease. Moreover, different cell types in the tumor microenvironment produced chemokines capable to recruit circulating T cells to the tumor site and cytokines capable to reprogram T cells to IL17 production. Tregs were decreased in the peripheral blood of SCC patients, but were significantly increased in the tumor compartment of these patients. Finally, we found that frequencies of infiltrating V2 T cells and Treg cells differently correlated with the tumor stage and ROC curve analysis suggest that the V2 Tcell/Treg ratio might be of diagnostic potential.

POSTER PRESENTATION

Martina Molgora

**IL-1R8: A NOVEL CHECKPOINT REGULATING ANTI-TUMOR AND ANTI-VIRAL ACTIVITY OF NK CELLS**

Humanitas Clinical and Research Center, via Manzoni 56, 20089 Rozzano (Milano), Italy

IL-1R8 is an Interleukin-1 receptor family member that acts as a negative regulator of IL-1 family receptor and TLR signaling. Both murine and human NK cells express high levels of IL-1R8 but its functional role in this cell type has not been described so far. Expression analysis showed that IL-1R8 was acquired during differentiation in human and murine
NK cells. IL-1R8 deficiency in the mouse was associated with higher frequency of mature NK cells, higher levels of activating NK cell receptors and increased Interferon-γ (IFN-γ), GranzymeB and Fas ligand expression and degranulation. IL-18, which is a key regulator of NK cell activities and can be targeted by IL-1R8, was responsible for this phenotype. Indeed, IL-1R8 regulated IL-18- MyD88 axis during NK cell differentiation and IL-18-dependent activation of mTOR and JNK pathways increased in IL-1R8-deficient NK cells. To assess the role of IL-1R8 in NK cells in pathology, we used models of MCA-induced lung metastasis and DEN-induced hepatocellular carcinoma. The number and dimension of lung metastasis and the liver disease severity were significantly reduced in Il1r8-/- mice. The depletion of NK cells in these models totally abrogated the protection observed in Il1r8-/- mice.

Finally, we investigated the role of IL-1R8 in NK cell antiviral activity, in a model of MCMV infection. Il1r8-/- mice controlled the virus more efficiently in liver and the protection was associated with enhanced NK cell degranulation and IFN-γ production. IL-1R8 plays a non-redundant role in the regulation of NK cell development and effector functions by tuning IL-18-dependent activities. IL-1R8 could be therefore a crucial regulator of NK cell antitumoral and antiviral potential.

POSTER PRESENTATION
Eleonora Fonte

TLR-9 STIMULATION INDUCES HETEROGENEOUS IKBZ EXPRESSION THAT PROTECTS CLL CELLS AND UNLEASHES IGM SECRETION
Cell Signaling Unit, Division of Experimental Oncology, IRCCS San Raffaele Hospital, Milano, Italy

Chronic lymphocytic leukemia (CLL) is the most frequent adult leukemia, characterized by the proliferation and accumulation of mature clonal BQlymphocytes in the peripheral blood and lymphoid tissues. CLL cells strongly depend on external stimuli for survival and proliferation. The best example is provided by the antigen receptor (BCR)as the inhibition of its downstream signaling pathways has shown clinical efficacy and led to approval of novel drugs for the treatment of CLL patients. Additional receptors and pathways are known to support leukemia development and progression, including cytokine receptors and coQstimulatory receptors such as TollQ Like Receptors (TLR). A better understanding of the molecules and pathways that may be involved in the proliferation and survival of the leukemic cells may help to fully understand CLL biology and design more effective targeting strategies.

Heterogeneity of response to different TLR ligands was previously observed in different patients being associated with their clinical course. In detail, distinct TLR ligands binding to TLR1/2, TLR2/6 and TLR9 induce costimulatory molecules in virtually all cases, but they can induce proliferation and chemoresistance only in a select subset of cases characterized by adverse prognostic markers (i.e. unmutated IGHV genes and CD38. Cytokines(i.e.TNFQβ)and distinct miRNAs are known to be selectively induced in aggressive IGHV unmutated cases but the molecular framework of the key signaling molecules leading to such variable response has yet to be determined.

We focused our studies on a subset of CLL cases characterized by expression of adverse prognostic
markers that respond to CpG, a TLR9 ligand, with enhanced cell survival. We analyzed the atypical IκBζ nuclear protein because it is specifically induced by TLR in different leukocyte populations but its role has never been characterized in CLL. Nevertheless, in the context of BQ cell types, it has been recently shown that IκBζ controls the proliferation of mouse B lymphocytes and triggers a TLR-dependent but TQ-independent antibody response.

We herein analyzed, for the first time, IκBζ expression regulation and function in leukemic cells from CLL patients. We observed that CpG stimulation induced IκBζ in leukemic cells; this upregulation was distinctively higher in a subset of CLL cases characterized by adverse prognostic markers. IκBζ protein was specifically upregulated after TLR9 ligation by an IRAK-dependent posttranscriptional mechanism in the group of CLL samples characterized by a TLR-induced proQsurvival program. Interestingly, IκBζ plays a causal role in sustaining CpG-induced cell survival and chemoresistance as demonstrated by siRNA mediated downregulation in CLL cells. TLR9 stimulation unleashed IgM secretion selectively in IκBζ positive cases through an autophagy-dependent mechanism. Moreover, ectopic overexpression of IκBζ in a CLL cell line demonstrated that IκBζ is causally involved in IgM secretion. Finally, expression analysis of IκBζ protein in normal and malignant cells showed a specific IκBζ overexpression induced by TLR9 in CLL cells.

These results provide novel insights into the pathobiology of CLL, and shed light onto the molecular pathways that mark and regulate aggressive CLL cases pointing to IκBζ as a central molecular regulator.

**BEST POSTER PRESENTATION**

Anna Pastò

**M1 MACROPHAGE POLARIZATION IS ASSOCIATED WITH CANCER STEM CELL EXPANSION**

Istituto Oncologico Veneto - IRCCS, Padova, Italy

Tumor growth, maintenance and relapse is controlled by a small population of cells called cancer stem cells (CSCs). Emerging evidence indicates that a pivotal role in tumor progression is played by inflammation, which creates both inhibitory and stimulatory conditions. The main effectors of such cross-talk between tumor and microenvironment are tumor-associated macrophages (TAMs) alternately activated into distinct phenotypic polarization, referred as M1 and M2. Recently we have demonstrated that p50 subunit of NF-kB is a key regulator of M2 polarization. We decided to investigate the effects of different macrophage polarizations on the CSC compartment in murine melanoma B16 cells. We demonstrated that ALDH could be a reliable marker for CSCs in this tissue. Indeed, ALDHpos cells were able to form spheroids in vitro under specific culture conditions and presented higher tumorigenic potential compared to ALDHneg cells.

Preliminary results showed that in p50(-/-) mice tumor growth was slower than in WT animals; however, ex vivo analysis revealed a higher percentage of ALDHpos cells. Moreover, when ex vivo isolated tumor cells from p50(-/-) mice were maintained for 1 week in vitro in stem culture conditions, a higher expression of stemness-related genes such as Nanog, Sox2 and Oct4, as well as a higher ratio of spheroid-forming cells, compared to tumor harvested from WT mice was observed.
Similar results were obtained when B16 cells were injected in LyzCre+ mice, lacking p50 in the myeloid compartment. Indeed, even in this model we observed a tumor growth slowdown, a higher expression of ALDHpos cells, and an increased expression of stemness-related genes after in vitro stem-enriched culture, compared to tumors obtained from control mice. The correlation between the lack of M2 polarization due to p50 knockout and CSC expansion was confirmed in in vitro experiments using tissue supernatants obtained from cells isolated from the different murine models utilized. Even in this setting, the lack of p50 was associated with an increase in the percentage of ALDHpos cells, as well as a higher expression of Nanog, Sox2 and Oct4. Our results indicate that M1 polarization is associated with an expansion of CSCs that seems to be time dependent. However, further experiments are needed to investigate how different polarized inflammatory programs control CSC expansion and differentiation and to identify myeloid-specific factors controlling the fate of CSCs.

POSTER PRESENTATION

Venera Russo

ADAPTIVE IMMUNE RESPONSES TRIGGERED BY HERPES SIMPLEX VIRUS TYPE 1 CAUSE ENTERIC NERVOUS SYSTEM DYSFUNCTIONS

Department of Molecular Medicine, 2 Department of Pharmacological Science, University of Padova, Padova, Italy

Introduction: Anomalies of the enteric nervous system (ENS) have been associated to motility and inflammatory disorders of the gut. Since enteric neuropathies are characterized by various hystopathological pictures including loss of ganglion cells or infiltrating mononuclear cells an immune-mediated damage, possibly triggered by an infectious agent, has been postulated. Indeed, we have recently shown in rodents that following intragastric (IG) administration the neurotropic Herpes simplex virus (HSV)-1 can infect the ENS resulting in time-dependent intestinal neuromuscular abnormalities1.

Aim: We aimed to investigate whether adaptive immune responses are involved in the onset of ENS dysfunction following HSV-1 infection

Methods: Male C57/B16 (WT) mice were inoculated intranasally with HSV-1 (102 pfu) and 4 weeks (W) later IG (107pfu). Starting 4 W after viral IG inoculum a group of mice received anti-CD8 antibody. Six-ten W after viral IG inoculum we: a) determined gastrointestinal (GI) motility by measuring fluoresceinisothiocyanate dextran distribution; b) assessed ENS integrity by immunohistochemistry on ileal wholemount preparations; c) characterized the lymphocytes infiltrating the longitudinal muscle myenteric plexus (LMMP) and their reactivity to HSV-1 antigens by FACS analysis; d) quantified changes in isometric muscle tension following electric field stimulation (EFS) of ileal segments.

To verify the role of lymphocytes in HSV-1 induced ENS dysfunction, CD3+ cells were isolated from the LMMP of mice 8-10 W after viral IG inoculum, in vitro pulsed with HSV-1, and injected in recipient mice. After one week the effects on isometric muscle tension following EFS stimulation of ileal segments were determined.

Results: At 8 and 10 W after IG inoculum, HSV-1 caused a significant delay in GI transit, impaired cholinergic neuromuscular transmission (p<0.01 vs control mice), altered expression and
distribution of the neurofilaments peripherin and βIII-tubulin, whereas no anomalies were observed at 6 W after IG inoculum.

In the LMMP, but not in the spleen, we observed an increase in CD3+ lymphocytes starting at 6 W and persisting up to 10 W after viral IG inoculum. At 8 W after viral IG inoculum HSV-1 reactive CD3+CD4+IL4+ and CD3+CD8+INF-γ+ were demonstrated in LMMP, whereas at 10 W non-HSV-1 specifically activated CD8+ T-cells were present in LMMP. Depletion of CD8+ T-cells, by administration of monoclonal anti-CD8 antibody, abolished the ENS damage and the neuromuscular anomalies observed at 8 W after viral IG inoculum. Ileal muscle tension was significantly altered (p<0.01 vs control) in recipient mice receiving in vitro HSV-1 pulsed LMMP lymphocytes isolated at the 8th W post IG viral inoculum.

Instead, the adoptive transfer of lymphocytes from 10 W infected mice resulted in dysmotility with or without in vitro exposure to viral antigens.

Conclusion: At different time points following infection of the ENS, HSV-1 activates lymphocyte subsets directly causing intestinal neuromuscular dysfunction. We speculate that HSV-1 infection of the ENS, as opposite to the central nervous system compartment, triggers adaptive immune responses that can alter the structure and the integrity of the neurons thus predisposing to bowel anomalies.

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**POSTER PRESENTATION**

Diana Saraiva

**ESTABLISHMENT OF A PROSPECTIVE STUDY TO UNVEIL THE PREDICTIVE VALUE OF BREAST CANCER IMMUNE FEATURES IN THE RESPONSE TO NEOADJUVANT CHEMOTHERAPY.**

CEDOC, NOVA Medical School, Faculdade de Ciências Médicas, Universidade Nova de Lisboa

Breast cancer (BC) is the most common cause of death in young women and its incidence is increasing as are the curability rates. BC is a heterogeneous disease and while some patients completely respond to neoadjuvant chemotherapy (NACT), others show minimal or no response. Thus, it is urgent to obtain more insights into predictors of the response to NACT regimens and elucidate which patients need alternative therapies.

The immune system plays a role in BC pathophysiology, with the presence of tumour infiltrating lymphocytes (TILs) and tumour associated macrophages (TAMs) being generally correlated with good and poor prognosis, respectively. Few studies have suggested that NACT modulates the immune infiltrate, increasing TILs, therefore positively impacting the prognosis. Still, this matter is not fully clarified, as the presence of TILs and TAMs only leads to limited information regarding tumour-destructive response. Indeed, effector lymphocytes could be poorly activated and/or tempered by the presence of regulatory cells and immunosuppressor molecules, influencing the response to NACT. Moreover, therapies based on immunologic response modifications have been tested in BC with low response rate, probably due to the lack of BC immune environment characterization and consequent patient misselection.

The aim of this work is the establishment of a prospective study to characterize the immune environment of a high number of breast cancer samples to find reliable immune features that would help to predict the response to NACT and to clarify which patients would benefit from
immunotherapies. We are analysing, by flow cytometry, the phenotype of immune cells (cytotoxic, helper and regulatory T lymphocytes, B lymphocytes, Natural Killer cells, dendritic cells, macrophages and neutrophils), their activation status and markers related with immunosuppressed tumour environment, within fresh BC tumour samples obtained pre- and post-NACT. We observed that pre-NACT samples are highly heterogeneous in terms of their lymphocytes and myelocytes subpopulations and their activation status, allowing a division of tumours into immune “hot” and immune “cold”. Interestingly, a positive correlation (p 0.01) was found between the activation of T regulatory lymphocytes and the expression of immunosuppressor molecules such as IDO and IL-10 and a negative correlation (p 0.01) was found between the number of macrophages and the expression of the inhibitory immune-checkpoints PD-1 and PDL1 and the immunomodulatory IDO and IL-10, by tumour cells. This highlights the idea that the immunogenicity of the tumour is beyond the quantity of TILs and TAMs found within the tumour. We intend to follow-up the immune features of these tumours after treatment and then correlate our data with patient outcome. Few samples post-NACT were analysed so far, but it seems that poor activation of effector lymphocytes and/or the presence of immunosuppressor molecules indeed contributes to bad response to NACT. Although preliminary, our results suggest that the heterogeneity of the immune environment is related with differences in patient response to NACT.

POSTER PRESENTATION

Matias Soncini

NFAT TRANSCRIPTION FACTORS, CANCER INITIATING CELLS AND CHEMORESISTANCE.

Dipartimento di Biotecnologie e Bioscienze, Università degli Studi di Milano-Bicocca

PGE2 is the most predominant prostaglandin, produced from arachidonic acid by the sequential action of PLA2, COX-2 and mPGES-1. It plays a role in several physiological processes, from central nervous system regulation to immune functions. In particular, it is known to be involved in classic signs of inflammation as redness, swelling and pain, and the CD14/NFAT pathway activation demonstrated to be necessary during edema formation for PGE2 synthesis by dendritic cells, by inducing the expression of mPGES-1. Recent studies demonstrated that human bladder cancer cells exposed to chemotherapeutic drugs produce PGE2, which promotes cancer initiating cells (CICs) re-activation and proliferation; this progressively leads to clinical chemoresistance in mouse models. Moreover, silencing studies on mouse breast cancer cell lines demonstrated that constitutively activated NFAT transcription factors intrinsically promote proliferation and metastasis, the latter, as well as chemoresistance, being a peculiar attribute of CICs, following cancer stem cells theory. The cancer stem cells theory was postulated after the description of discrete small pools of cells, in several solid and hematopoietic malignancies, capable of initiate and sustain tumors. CICs are characterized by stem-like properties as self-renewal and capability to regenerate (tumor) tissue. The NFAT transcription factors have been originally described as fundamental mediators of IL-2 expression in T cells; since then multiple NFAT functions have been progressively identified in both adaptive and innate immunity. Beside the roles described in immune cells infiltrating tumors, NFAT family members were found to have a deregulated expression or presented nuclear accumulation in some human solid tumors and hematologic
malignancies. In preliminary experiments, we observed, on a murine breast cancer cell line, that cisplatin treatment can increase NFAT translocation. This could explain the increased production of PGE2 observed during chemotherapy. The expression of VIVIT peptide, capable of inhibiting NFAT activation by blocking the interaction between NFAT and Calcineurin, blocks the Cisplatin-dependent NFAT translocation and reduce the basal levels of Cox2 and mPges1, leading to a reduced PGE2 production in the same cell line. The supernatant derived from VIVIT-expressing, Cisplatin-treated cells, is less capable of inducing CICs proliferation in an in vitro spheroid-assay. Moreover, we observed that the same VIVIT-expressing cells presented a reduced number of Sca1+ cells and generated a reduced number of mammospheres, suggesting also an intrinsic role of NFAT in CICs maintenance. A Tail-Vein injection experiment supports this hypothesis in vivo: VIVIT-expressing cells injected i.v. gave origin to less metastatic foci in lungs compared to WT. The confirmation of the role of NFAT in cancer cells stemness will eventually define a novel family of drugs to be used as supplementary therapy, in combination with chemotherapy or tumor explant, to reduce the pool of CICs and their re activation by PGE2, minimizing the risk of both metastasis and relapse.

**POSTER PRESENTATION**

Matias Soncini

**24-HYDROXYCHOLESTEROL PARTICIPATES IN PANCREATIC NEUROENDOCRINE TUMOR DEVELOPMENT**

Dipartimento di Biotecnologie e Bioscienze, Università degli Studi di Milano-Bicocca

Cells in the tumor microenvironment may be reprogrammed by tumor-derived metabolites. Cholesterol oxidized products, namely oxysterols, have been shown to favor tumor growth directly by promoting tumor cell growth and indirectly by dampening antitumor immune responses. However, the cellular and molecular mechanisms governing oxysterol generation within tumor microenvironments remain elusive. We have recently shown that tumor-derived oxysterols recruit neutrophils endowed with protumoral activities, such as neoangiogenesis. Here, we show that HIF 1α controls the over-expression of the enzyme Cyp46a1, which generates the oxysterol 24 hydroxycholesterol (24-HC) in a pancreatic neuroendocrine tumor (pNET) model commonly used to study neoangiogenesis. The activation of the HIF-1α-24-HC axis ultimately leads to the induction of the angiogenic switch through the positioning of pro-angiogenic neutrophils in proximity to Cyp46a1+ islets. Pharmacologic blockade or genetic inactivation of oxysterols controls pNET tumorigenesis by dampening the 24-HC-neutrophil axis. Finally, we show that in some human pNET samples Cyp46a1 transcripts are over-expressed, which correlate with the HIF-1α target VEGF and with tumor diameter. This study reveals a new player in the angiogenic switch of pNETs and identifies a new therapeutic target for pNET patients.

**POSTER PRESENTATION**

Nabila Tounsi
CONTRIBUTION OF ADENOSINE DEAMINASE AND XANTHINE OXIDASE ACTIVITIES IN OVARIAN CANCER TUMOURIGENESIS AND MALIGNANCY
University of sciences and technology Houari Boumedienne, Algiers, Algeria

Introduction: Ovarian cancer is the 7th most common cancer in women with 238719 new cases and 151917 deaths in 2012. In Algeria, the ovarian cancer is the 4th most common cancer with worse prognosis for more than 66% of new cases 821 dies in 2012. The goal of our study was to evaluate the contribution of serum adenosine deaminase (ADA) and xanthine oxidase (XO) as purine catabolism markers in Algerian ovarian cancer patient’s progression.

Materials and Methods: Hematoxylin and eosin (H&E) stained sections of ovarian tumors were reviewed for diagnosis and benign or malignant tumor determination. Sera from 40 ovarian cancer patients before treatment among of them, 30 malignant tumor and 30 controls were sampled and used for seric ADA and XO activities evaluation.

Results: H&E stained sections of ovarian cancer showed that among the 40 patients, 30 are malignant tumor and 10 benign tumor. The 30 malignant tumor displayed 2 patients in stage1, 12 patients in stage 2, 11 patients in stage 3 and 4 patients in stage 4. ADA activity increased by 50% (p < 0.001) and XO activity by 40% (p < 0.001) in malignant ovarian cancer patients, compared to healthy individuals. Significant differences were shown between serum ADA and XO activities of benign and malignant ovarian cancer patients. Ovarian cancer patients had significant elevated seric levels of ADA and XO activities, which was associated to tumoral stage progression.

Conclusion:
Our preliminary data show that purine catabolism by enhanced ADA and XO activities under cancerous conditions may create a supportive environment for ovarian cancer progression. The potential of targeting purinergic pathways can provide a new immunotherapeutic treatment of ovarian cancer.

Keywords: Adenosine deaminase, purine catabolism, ovarian cancer, xanthine oxidase

BEST POSTER PRESENTATION
Mirko Trilling

CMV PARTICLES MEDIATE ACUTE MYELOID LEUKAEMIA CELL DEATH VIA SOLUBLE FACTORS IRRESPECTIVE OF VIRAL REPLICATION, GENE EXPRESSION, AND ENTRY
Institute for Virology, University Hospital Essen, University Duisburg-Essen, Essen, Germany

Acute myeloid leukemia (AML) is caused by malign transformations of cells from the myeloid lineage leading to their uncontrolled proliferation and subsequent malfunction of hematopoiesis. Bone marrow ablation and subsequent reconstitution e.g. by hematopoietic stem cell transplantation (HSCT) is a frequently used treatment option with curative potential. Although HSCT recipients exhibit exaggerated vulnerability towards several infectious agents due to the ablation of the immune system and immunosuppressive therapy, a surprising correlation between early (<d100) human cytomegalovirus (HCMV) reactivation and reduced AML relapse has been documented. Based on the absence of this correlation in case of lymphocytic malignancies as well as HHV-6, HSV, VZV, and EBV infections, we infer that an association between HCMV, its tropism, and reduced AML relapse might exist.

Studying this correlation, we observed that very low multiplicities of HCMV infection cause overt death of several AML cell lines. Boiling or sterile filtration of the HCMV inoculum abrogated the
effect, but it was still evident upon treatment with pan-caspase or necroptosis inhibitors. Furthermore, the effect was also observed in presence of Ganciclovir and upon contact with UV-irradiated HCMV virions. Consistent with a dependency on the pentameric entry complex, HCMV strain TB40/E - but not strain AD169 - entered AML cells as determined by pIE1-pp72 expression. However, both strains equally inhibited the proliferation of several AML cell lines and induced AML cell death. We concluded that HCMV particles elicit this detrimental effect on AML cells irrespective of viral replication, gene expression and entry. Consistent with the results of low MOI infections, sterile supernatants from HCMV-experienced AML cells killed fresh AML cells. Based on the finding that such supernatants activate NF-κB and GAS reporter cells, we currently study if HCMV particles stimulate AML cells to induce a suicidal innate immune response.

TOPIC: OTHER

BEST POSTER PRESENTATION

Massimo Aureli

Plasma membrane response to P. aeruginosa infection: from molecular mechanisms to therapeutic strategies for cystic fibrosis lung inflammation

Department of Medical Biochemistry and Translational Medicine, University of Milano, Italy

Several studies indicate that sphingolipids (SL) play a regulatory role in inflammatory response ranging from bacterial-cell interaction to autoimmunity. In cystic fibrosis (CF) they are directly involved in the in airway inflammation responsible for the onset of CF lung disease. In particular, ceramide derived from glycosphingolipids (GSL) has gained more interest since inhibition of the glucocerebrosidase GBA2 and its down-regulation by siRNA, are associated with a significant reduction of IL-8 secretion after P. aeruginosa (PAO) infection, as well as a reduction of the intrinsic inflammatory state in human CF epithelial bronchial cells (CFhEBC). We hypothesized that the aberrant inflammatory response to PAO in CFhEBC starts from alterations in the lipid composition of specific plasma membrane (PM) macromolecular complex by the action of the GSL-hydrolases associated with the cell surface, including GBA2. To figure out the possible molecular mechanism, we investigated the effect of PAO infection on specialized membrane area called lipids rafts. Moreover, with the aim to develop a new anti-inflammatory treatment, we tested new lipid based-nanoparticles for the delivery of siRNA targeting GBA2. The data obtained further support the role of GBA2 in the inflammatory response upon PAO infection. Moreover, we found that in CFhEBC PAO causes a recruitment of PM-associated GSL-hydrolases into lipids rafts. At this site, the enrichment of enzymes involved in GSL catabolism causes a reduction of the ganglioside GM1 and sphingomyelin, which is paralleled by increased levels of Glucosylceramide and Ceramide, both events responsible for the activation of the inflammatory response. In addition, we developed lipid-based non-viral nanovectors (NP) able to silencing GBA2 in vitro for up 8 days with any toxicity for the cells. By X-ray analysis, we identified the most promising NP structure in order to penetrate CF mucus. Overall our results suggests that the identification of GBA2 as well as other GSL-hydrolases as possible molecular targets related to the inflammatory response and the development of an innovative NP-based approach for their silencing could represent an important
amelioration of the therapeutic strategies for several immune-derivative disease, including CF.

BEST POSTER PRESENTATION

Monica Cappelletti

TYPE I INTERFERONS REGULATE SUSCEPTIBILITY TO INFLAMMATION-INDUCED PRETERM BIRTH
Divisions of Immunobiology, Cincinnati Children’s Hospital Research Foundation, and the University of Cincinnati
College of Medicine, Cincinnati, OH 45229, USA. #Immunology Graduate Program and $Molecular, Cellular and Biochemical Pharmacology Graduate Program, University of Cincinnati College of Medicine, Cincinnati, OH 45220

Preterm birth (PTB) is a leading worldwide cause of morbidity and mortality in infants. Maternal inflammation induced by microbial infection is a critical predisposing factor for PTB. However, biological processes associated with competency of pathogens, including viruses, to induce PTB or sensitize for secondary bacterial infection-driven PTB are unknown. We show that pathogen-/pathogen associated molecular pattern (PAMP)-driven activation of type I Interferon (IFN)/IFN receptor (IFNAR) was sufficient to prime for systemic and uterine proinflammatory chemokine and cytokine production and induction of PTB. Similarly, treatment with recombinant type IFNs recapitulated such effects by exacerbating proinflammatory cytokine production and reducing the dose of secondary inflammatory challenge required for induction of PTB. Inflammatory challenge driven induction of PTB was eliminated by defects in type I IFN, TLR or IL-6 responsiveness, whereas the sequence of type I IFN sensing by IFNAR on hematopoietic cells was essential for regulation of proinflammatory cytokine production. Importantly, we also show that type I IFN priming effects are conserved from mice to non-human primates and humans, and expression of both, type I IFNs and proinflammmatory cytokines is upregulated in human PTB. Thus, activation of the type I IFN/IFNAR axis in pregnancy primes for inflammation-driven PTB and provides an actionable biomarker and therapeutic target for mitigating PTB risk.

BEST ORAL PRESENTATION

Elena Ciaglia

NK CELLS IN HUMAN BRAIN TUMOR CONTROL: EVIDENCE OF A DISTINCT PHENOTYPIC AND FUNCTIONAL PATTERN IN TUMOR VS NON TUMOR INFILTRATED BRAIN.
Department of Medicine, Surgery and Dentistry, University of Salerno, Baronissi Italy

Introduction:
Probably because the CNS has long been considered an immune-privileged site not able to generate a classical immune response, the capacity of CD56+ NK cells in exerting beneficial effects against Glioblastoma (GBM) has been remained understudied.

Methods
To investigate the role of NK cells in disease progression, we analyzed frequency, phenotype and functions of NK cells from tumor-infiltrated (TIB), tumor-free peripheral (TFB) brain and peripheral blood (PBL) in a cohort of stage III-IV glioma patients.

Results and Conclusions
The comparative FACS analysis of the lymphocytes subsets associated with different regions of GBM cell suspensions and autologous PBL reveal a dynamic nature of NK cell subpopulation, potentially reflecting tumor biology. Compared with tissue from tumor margins and blood, TIB are enriched of resting cytokine-producing CD56bright NK cells, specifically retained via the engagement of CCR5 pathway. Here, the proteome analysis also shows a cytokine milieu (IL-6, IL-13, M-CSF, GM-CSF, angiopoietin, MIP, MCP, VEGF) suggestive of a dysregulation of mielomonocytic lineage toward a pro-tumor phenotype. On the contrary, TFB-NK are terminally differentiated (CD56dim, CD57+CD107a+CXCR3+) but in a resting state (CD16-, KIR low, CD69-, NKG2D-).

In conclusion our data suggest that microenvironment of brain generates and/or recruits different NK cell subsets. Interestingly, the phenotypic and functional differences between TIB- and TFB-NK cells, critical to distinguish specific innate immune responses against brain tumors, will be useful to design new immunotherapeutic approaches.

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BEST POSTER PRESENTATION

Marina Gazdic

MESENCHYMAL STEM CELLS ATTENUATE ACUTE LIVER INJURY BY ALTERING RATIO BETWEEN IL-17 PRODUCING AND REGULATORY NKT CELLS

Centre for Molecular Medicine and Stem Cell research, Faculty of Medical Sciences University of Kragujevac Serbia

Mesenchymal stem cells (MSCs) are, due to immunomodulatory characteristics, considered as novel agents in the treatment of immune-mediated acute liver failure. Although it is known that MSCs can regulate activation of T lymphocytes, their capacity to modulate function of neutrophils and NKT cells, major interleukin (IL)-17 producing cells in acute liver injury, is still unknown.

By using two well established murine models of neutrophil and NKT cell mediated acute liver failure (induced by Carbon tetrachloride (CCl4) and alphagalactoceramide (α-GalCer)), we investigated molecular and cellular mechanisms involved in MSC-mediated modulation of IL-17 signaling during acute liver injury.

Single intravenous injection of MSCs attenuate acute hepatitis and hepatotoxicity of NKT cells in paracrine, indoleamine 2,3-dioxygenase (IDO)-dependent manner. Decreased levels of inflammatory IL-17 and increased levels of immunosuppressive IL-10 in serum, reduced number of IL-17 producing NKT (NKT17) cells and increased presence of FoxP3+ IL-10 producing NKT regulatory (NKTreg) cells were noticed in the injured livers of MSC-treated mice. MSCs did not significantly alter total number of IL-17-producing neutrophils, CD4+ and CD8+ T lymphocytes in the injured livers. Injection of MSC conditioned medium (MSC-CM) resulted with increased NKTreg/NKT17 ratio in the liver and attenuated hepatitis in vivo and significantly reduced...
hepatotoxicity of NKT cells in vitro. This phenomenon was completely abrogated in the presence of IDO inhibitor, 1-methyltryptophan.

In conclusion, the capacity of MSCs to alter NKT17/NKTreg ratio and suppress hepatotoxicity of NKT cells in IDO dependent manner may be used as new therapeutic approach in IL-17 driven liver inflammation.

Keywords: Mesenchymal stem cells, acute hepatitis, NKT cells, IL-17, regulatory cells.

BEST POSTER PRESENTATION

Gieryng A.

KNOCKDOWN OF OSTEOPONTIN (SPP1) IN GLIOMA CELLS BLOCKS PROTUMORIGENIC ACTIVATION OF INFILTRATING MYELOID CELLS AND RESTORES ACTIVATION OF CYTOTOXIC T CELLS

Laboratory of Molecular Neurobiology, Neurobiology Center, The Nencki Institute of Experimental Biology, Warsaw, Poland

Background. Human malignant gliomas are infiltrated with myeloid cells, especially microglia and peripheral macrophages, and lymphocytes. Despite their accumulation and activation in tumor microenvironment, anti-tumor immune responses are defective in these tumors. Tumor-educated immune cells induce an immunosuppressive environment and paralyze cytotoxic T (Tc) cells, support glioma invasion and progression. Osteopontin (Spp1, secreted phosphoprotein 1), a potent immune cell attractant and activator, is secreted and processed by glioma cells. Tumor derived Spp1 induces glioma-associated activation of microglia/macrophages thereby supporting glioma progression. In this study, we analyzed the effects of Spp1 knockdown in glioma cells on the response of immune cells infiltrating intracranial gliomas.

Material and Method. Lentivirally delivered shRNA were used to knockdown Spp1 in glioma cells and Spp1 stably depleted cell lines were developed. Silencing of Spp1 did not affect cell proliferation and clonogenic potential (MTT, BrdU and clonogenic assaya). Glioma shSpp1 and shNeg cells were intracranially implanted to Wistar rats and 14th or 21st days later CD11b+ and CD8+ cells infiltrating gliomas were sorted and tumor volume was measured. Transcriptome profiling with Affymetrix Rat Gene 2.1 ST microarray and quantitative RQ-PCR were performed. Results and discussion. Spp1 knockdown did not affect accumulation of resident microglia, blood-derived macrophages and leukocytes in gliomas but significantly reduced tumor growth. However, the computational analysis of gene expression revealed different profiles in sorted CD11b+ cells from control and Spp1 depleted gliomas. Significant differences in clusters related to DNA replication, translational regulation, mitosis and defence response, immune response were detected. Moreover, the expression of genes characteristic for protumorigenic activation was reduced in Spp1 depleted gliomas. Detected differences were verified by RQ-PCR and we found Nos2, Ccl2 and Arg1, Mmp14, Id3, Tgm2 mRNAs reduced in CD11b+ cells from Spp1 depleted gliomas. Detailed analysis of the expression of transcription factors characteristic for T-cell subpopulations and markers of activated Tc cells showed restoring of anti-tumor responses in sorted CD8+ cells from Spp1 depleted gliomas. Conclusions: Our results confirm a critical role of tumor-derived Spp1 in shaping glioma microenvironment and suggest the impaired acquisition of protumorigenic phenotype in CD11b+ cells from Spp1 depleted gliomas. The presence of activated Tc cells could be a sign of restoration of the antitumor activity.
of immune responses in SPP1-depleted gliomas, which results in tumor eradication. Supported by
2012/04/A/NZ3/00630 grant from the National Science Center.

POSTER PRESENTATION

Angela Gupta

CYTOKINE TOLERANCE IN ASTROCYTES: RELB-DEPENDENT EPIGENETIC SILENCING LIMITS NEUROINFLAMMATION

Virginia Commonwealth University, Department of Biochemistry, Richmond, VA

Astrocytes play a critical role in modulating inflammation in the central nervous system. In
diseases such as multiple sclerosis or cancer where there are high levels of neuroinflammation,
astrocytes become reactive, undergo dramatic morphological and functional changes, and both
secrete and respond to a host of inflammatory mediators. However to avoid damage associated
with chronic inflammation in the brain, astrocytic inflammatory responses need to be tightly
regulated. A key hallmark of cells of the monocyte-macrophage lineage is tolerance; a homeostatic
response to repeated stimuli that prevents excess inflammation. Nevertheless, the question
remains whether astrocytes also display similar adaptive responses. We have established that
primary human astrocytes exposed to proinflammatory cytokines such as IL-1 develop days-long
“cytokine-tolerance” to subsequent cytokine activation. This adaptive component provides
“memory” or “imprinting” of astrocytes, which resembles endotoxin-induced tolerance observed
in macrophages. Mechanistically, we found that “cytokine-tolerance” in astrocytes depends on
induction of RelB expression, a unique member of the NF-κB family, its phosphorylation, and
subsequent RelB-dependent epigenetic silencing of cytokine genes. These epigenetic changes of
cytokine genes diminish further expression, and therefore limit inflammatory responses. To
further examine the role of RelB in neuroinflammation in vivo, we have generated astrocyte-
specific (GFAP-cre) and neural progenitor-specific (Nestin-cre) RelB knockout mice. We have
induced experimental autoimmune encephalomyelitis (EAE) in these mice to determine the role
of astrocytic RelB in neuroinflammation.

POSTER PRESENTATION

Monire Jahantigh

ALLOGENEIC NON-ADHERENT BONE MARROW CELL-DERIVED MESENCHYMAL STEM CELLS IN AMELIORATING RAT MODEL OF ULCERATIVE COLITIS

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Introduction: Recently, it has been demonstrated, non-adherent bone marrow derived cells (N-
MSCs) can differentiate into various mesodermal cell lineages similar to other classic adherent
mesenchymal stem cells (A-MSCs). This study was done to investigate the anti-inflammatory and
regenerative capacities of allogeneic N-MSCs therapy in ameliorating rat model of ulcerative
colitis.
Method and materials: N-MSCs and A-MSCs from Wistar rats were separated and cultured using the pour-off method and classic method (frequent medium change in primary culture and diminishing the trypsinization time), respectively. Ulcerative colitis was induced in the male Wistar rats by luminal instillation of acetic acid. Animals in the treatment groups received N-MSCs or A-MSCs (2^−106 cell- i.p.) after acetic acid instillation. Animals were monitored for 10 consecutive days.

Results: Flow cytometric data showed that the marker for hematopoietic cell (CD 45) was absent in both cell N-MSCs and A-MSCs whereas consensus markers for MSCs of rat (CD 29 and CD 90) were clearly expressed. Nevertheless, the levels of CD 29 and CD 90 marker in A-MSCs were slightly lower than NMSCs. The animal examinations showed that both therapies with could regress the clinical scores, the mortality rate and gross pathology of ulcerative colitis in a comparable manner. Both therapy reduce the levels of the levels of malondialdehyde, myeloperoxidase, nitric oxide, and the concertation of TNF-س  س -IL-1 and IL-6, myeloperoxidase activity were down-regulated in the colonic tissues of treated rat without any significant difference.

Discussion: As isolation of non-adherent MSCs is very rapid and simple, this approach may be as a useful strategy to control colitis.

BEST POSTER PRESENTATION

Marco Pio La Manna

QUANTITATIVE AND QUALITATIVE PROFILES OF CIRCULATING MONOCYTES MAY HELP IDENTIFYING TUBERCULOSIS INFECTION AND DISEASE STAGES

Central Laboratory of Advanced Diagnosis and Biomedical Research (CLADIBIOR)

Tuberculosis (TB) is one of the most important cause of morbidity and death among infectious diseases, and continuous efforts are needed to improve diagnostic tools and therapy. Diagnosis of active TB disease still represents a challenge for the clinical management. Interferon-γ Release Assays (IGRAs) cannot distinguish between subjects with latent infection (LTBI) and active TB disease, and are inadequate for monitoring treatment response; moreover they also poorly predict those infected individuals who will progress to active TB. Previous published studies showed that the absolute number of monocytes or lymphocytes in peripheral blood or yet the ratio of monocytes to lymphocytes displayed the ability to predict the risk of active TB.

We evaluated the ratio of monocytes to lymphocytes and we the ex-vivo expression of CD64 on monocytes, as tools to identify biomarkers for TB stages discrimination. A total of 173 individuals were enrolled as here reported: (a) Healthy Donors (HD): 31 individuals tested TST and QFT-IT-negative; (b) LTBI subjects: 37 individuals QFT-IT-positive, with negative chest x-Ray results for active pulmonary lesions and no prior preventive therapy performed; (c) active TB disease: 71 individuals diagnosed with active pulmonary TB (with a positive Mtb culture) (d) 34 cured TB patients. Full blood counts (FBC) of peripheral blood collected in EDTA containing tubes were performed by one clinical diagnostic laboratory using a five-part differential hematology analyzer. Surface staining of PBMC was performed on freshly drawn whole blood using mAbs to CD14, CD64, CD16, CD123, CD152, CD163, CD206, HLA-DR, CD3, CD19, CD56 and their isotype controls. Four-parameter FCM acquisition and analysis were performed on a two-laser FACSCalibur
instrument using CellQuest software. The median or geometric mean was used for descriptive statistics for each parameter. The non-parametric Kruskal-Wallis was performed comparing the medians of ML ratio and the relationship between variables was evaluated using Spearman rank correlation test, p<0.05 was considered statistically significant. Data were analyzed using the Statistic software and GraphPad prism, version 5.0. Significant differences were found when the average ratio of monocytes to lymphocytes of active TB patients was compared with latent TB infection (LTBI) subjects, cured TB and healthy donors. By the receiver operator characteristics (ROC) curve analysis the cut-off value of 0.285, allowed the discrimination of active TB from HD groups, with a sensitivity of 91.04% and a specificity of 93.55%. The ROC curve analysis comparing TB patients and LTBI groups, led to a sensitivity and the specificity of the assay of 85.07% and 85.71%, respectively. Moreover, upregulation of CD64 expression on circulating monocytes in active TB patients could represent an additional correlate of active TB. In conclusion, we found that the evaluations of absolute number and phenotype of monocytes can be instrumental for TB stages identification.

BEST POSTER PRESENTATION

B. Neili

AUTOPHAGY INTERFERE IN THE REGULATION OF THE IMMUNE RESPONSE IN CHRONIC INFLAMMATORY BOWEL DISEASE

Laboratory of genetics, Immunology and Human Pathologies, Faculty of Sciences of Tunis, University Tunis El Manar, Tunis, Tunisia

PURPOSE

Study of the function of regulatory T cells (Treg) in the chronic inflammation and evaluate the role of autophagy in the modulation of the immune response.

METHODS

The cytometric analysis was performed to evaluate the percentage of Tregs CD4+ CD25hi in peripheral blood of Inflammatory Bowel Diseases (IBD) patients as well as to estimate the rate of Treg apoptosis. Two populations were tried Tregs and Teffectors using BD FACS Aria sorter, the cells were stained with antibodies for sorting. Tregs and Teffectors recovered after sorting were counted and re-suspended in appropriate media for each population in two different conditions. The suppressive activity of expanded Tregs was assessed in vitro by performing suppression of proliferation assay, T effector cells were stained with 5 Mm carboxyfluorescein diacetate succinimidyl ester (CFSE) and plated in co-cultures with expanded Treg cells in different proportions. As positive and negative control, T effector cells were used cultured with or without beads, respectively. After 4 days of culture, cells from each well were collected and CFSE dye dilution was measured. With the same sample we proceeded to RNA extraction using Trizol protocol, then we performed real time PCR to evaluate the autophagic activity in each type of cells (ATG5, P62, LC3II genes).

RESULTS

The cytometric analysis reveals that the Tregs cells are present with the same rate in both groups of study healthy donors and patients. Then the suppression test shows that the expanded T regs were unable to suppress proliferation of T effectors in IBD patients. The real time PCR performed using the autophagy markers and demonstrates that autophagy activity is reduced in IBD patients.
and especially the ATG5 gene is down regulated in comparison with T effectors

CONCLUSION

Those experiments are showing that unbalanced immune response in IBD is the result of cumulative factors which are important to maintain homeostasis of the immune system and autophagy is necessary for activation of T cells and prolongation of lymphocyte survival as demonstrated for ATG5 which is important for T cells to be activated.

POSTER PRESENTATION

Ferdinando Oriolo

IDENTIFICATION OF A NOVEL SUBSET OF HUMAN INTRA-EPITHELIAL NKp46+/Vd1 T CELLS RESIDENT IN THE INTESTINAL MUCOSA UNDER HOMEOSTATIC CONDITIONS

Unit of Clinical and Experimental Immunology, Humanitas Clinical and Research Center, Rozzano, Milan, Italy; Department of Medical Biotechnologies and Translational Medicine (BioMeTra), University of Milan, Milan, Italy.

Background: Human intestinal immune system constitutes the largest immune compartment of the body and plays a critical role in the maintenance of the gut homeostasis. In particular, highly heterogeneous populations of intraepithelial lymphocytes (IELs) are essential both to maintain the epithelial barrier’s integrity and, at the same time, to protect against the invasion of potential pathogens. On the other hand, their excessive immune activation has to be highly controlled to avoid unnecessary inflammatory responses. Gamma-delta (gd) T cells constitute a significant proportion of all antigen-experienced intestinal IELs and can reach about 20% of total lymphocytes compared to a lower degree 5% of human circulating T cells. gd T cells are a conserved class of lymphocytes; however, contrary to the well-studied ab T cells, their tissue specific distribution as well as their phenotypic characterization has not been fully characterized yet.

Recently it has been observed that human circulating gd T cells upon in vitro IL-2 and TCR engagement stimulation acquire highly anti-tumor and anti-viral activity associated to the expression of Natural Cytotoxic Receptors (NCRs) which include NKP30, NKP44 and NKP46. Historically NCRs has been identified as a specific Natural Killer (NK) cell receptors that trigger NK cell cytotoxicity and production of inflammatory cytokine against harmful cells. Therefore, we wondered whether this in-vitro NCRs positive phenotype achievement may be related to the physiological and tissue-specific adaptation/activation of human gd T cells.

Results: Multiparametric 6ow cytometry analysis of human normal colon mucosa, obtained from patients who underwent surgical resection, allowed the identification of a novel gd T cell subset expressing NKp46 receptor. NKp46pos/gd T lymphocytes prevalently resident in the intraepithelial compartment (average of 35% of all gd T IELs) rather than in lamina propria (LP) (average of 10% of all gd T LPLs) and are phenotypically distinct from gd T lymphocytes of LP. Indeed, they are characterized by highly tissue-diAerentiated and cytotoxic-like phenotype as a result of expression of diAerent plasma membrane markers such as 2B4, CD69, CD103 and CD56, CD8, NKG2D, NKG2C respectively. In addition, ex-vivo isolated NKp46pos/gd T IELs cultured with human tumor K562 cells results in an increased anti-tumor killing eDciency compared to NKp46neg/gd T counterpart. Expression of NKp46 in intestinal gd IELs is restricted to Vd1 TCR chain repertoire that distinguishes this subset from peripheral blood gd T cells which mainly exhibited a Vd2 TCR distribution. Interestingly, cytokine-induced diAerentiation of human gd T thymic precursor also
induces a de-novo expression of NKp46. This NKp46pos phenotype was selectively acquired upon IL-2 and IL-15 stimulation and it was associated with high cytolytic activity against diAerent human cancer cell lines. In addition, human intestinal epithelial cells and soluble factors such as IL-10 and TGF-b can modulate the NKp46 expression suggesting a possible gut-conditioned diAerentiation/maturation of NKp46pos/gd T cells.

Conclusion: We identified a novel NKp46pos/gd T lymphocytes subset resident in the human intestine likely relevant both in GALT homeostasis and physiopathology of the gut.

BEST POSTER PRESENTATION

Valentina Orlando

REGULATORY ATYPICAL B CELLS ARE EXPANDED IN PATIENTS WITH ACTIVE TUBERCULOSIS.

Central Laboratory Advanced Diagnosis and Biomedical Research (CLADIBIOR)

Introduction
Tuberculosis (TB) is the major infectious cause of death and morbidity worldwide, with about 10 million of new cases reported each year. TB is transmitted by inter-human contact following inhalation of cough aerosols carrying pathogens of the Mycobacterium tuberculosis (M. tuberculosis). The majority of the individuals infected with M. tuberculosis contain the infection and remains asymptomatic, defined as latent TB (LTBI), and only the 5-10% of them will develop active TB disease in their lifetimes. Control of the TB epidemic it has not yet reached because of the lack of an effective vaccine and of rapid and sensitive diagnostic approaches, as well as the emergence of drug-resistant forms of M. tuberculosis. While cellular immunity is fundamental in the containment of the infection, the role of humoral immunity is not yet clear.

In this study we have analyzed the frequency of B cells, their phenotype and functional features, focusing in particular on the balance between regulatory B cells (Breg) and proinflammatory B cells (Binf), distinguished by their capacity to express IL-10 or granulocyte macrophage-colony stimulating factor (GM-CSF).

Material and methods
PBMC obtained from LTBI subjects, active TB patients and healthy donors (HD), were stimulated in complete RPMI with ionomycin and PMA for 6 hours, in the presence of monensin for the last 5 hours. After the incubation, the cells were surface stained with anti-human CD19, anti-IgD and anti-CD27 (BD Biosciences) and intracellularly stained with anti-human IL-10 and anti GM-CSF (BD Biosciences). For each sample 100,000 viable cells were acquired on a FACS Canto II flow cytometer (BD Biosciences). Data were analyzed using FlowJo software version 9 (Treestar, Ashland, OR). Finally statistical analysis was performed by Graph Pad software using Mann Whitney test.

Results
A lower frequency of B-lymphocytes was found in active TB patients (3.96%) than in HD (5.97%) and LTBI subjects (5.73%), although differences did not attain statistical significance. Phenotypic distributions of B cell subsets (naive IgD+CD27-, IgM memory IgD+CD27+, switched memory IgD-CD27+ and atypical IgD-CD27-), did not display significant differences in the three tested groups. We have observed an increase of the ratio Breg/Binf in active TB compared to HD caused by an
slight increase of the percentage of IL-10+ B cells and a decrease of the percentage of GM-CSF+ B cells. Among the subsets, we have found a significant alteration of this ratio only at the level of two subsets, memory and atypical B cells, with a significant reduction of the ratio in the IgM memory subsets of active TB respect to those of HD, due to a remarkable decrease of percentage of IL10+ B cells and an increase of the percentage of GMCSF+ B cells, while in atypical B cell subsets the increase of this ratio in the active TB compared with HD is mainly due to the increase of the IL10+ B cells.

Conclusions
Our findings indicate an altered ratio of the frequencies of Breg and Binf in the IgM memory and atypical B cell subsets of active TB patients which is involved in host immunity during *M. tuberculosis* infection.

**POSTER PRESENTATION**
R. Toumi

**LACTOBACILLUS AND BIFIDOBACTERIUM TREATMENT REGULATE IFN-Γ AND TLR-4 EXPRESSION I (DSS)-INDUCED COLITIS MOUSE MODEL.**
Laboratory of Cellular and Molecular Biology (LBCM), Cytokines and NOSynthases

Inflammatory Bowel Diseases (IBD) including Crohn diseases (CD) and Ulcerative colitis (UC) are chronic conditions that involve inflammation of the gastrointestinal tract. The exact etiology of IBD remains unclear with no treatment able to achieve a complete healing. Several hypotheses suggest that IBD results from an abnormal immune response against endogenous flora and luminal antigens in genetically susceptible individuals. Because of the dysbiosis and the overall decrease in intestinal microbial diversity and stability of patients, manipulation of the gut flora with probiotics represents a promising alternative or complementary approach to the conventional treatment of IBD. In our study, we were interested in evaluating the potential use of a probiotic cocktail containing four live bacterial strains: *L. acidophilus, L. plantarum, B. lactis* and *B. breve* in the induction and maintenance of remission during Dextran sulfate induced colitis (DSS) in BALB/c mice.

We attempted to investigate the mechanisms involved in maintaining the integrity and inducing recovery of the intestinal epithelium. The DSS+Prb groupe is the Probiotic treatment group received 3% (w/v) of DSS for 5 days and 109 CFU/ml of probiotic for 7 days starting from day 3 to the end of the experimental period. A disease activity index (DIA) scores was used to assess body weight loss, stool consistency, and rectal bleeding. The concentrations of IFN-γ in plasma were measured by ELISA kit. We also investigated TLR-4, NF-κB and NOS-2 expression. Our results showed that Probiotics decreased the pro-inflammatory response (decreasing the expression of TLR-4, NF-κB, NOS-2, IFN-γ). These results suggest that probiotics used in our study contribute to the orientation of the immune response to an immunoregulatory profile required for the resolution of inflammation and the induction of remission. Probiotic supplementation to colitic mice after the onset of colitis and during the recovery period has significantly reduced production of NO in plasma. Probiotics could be considered as potent alternative and supplementary therapy approach in IBD which target both the microbiota and the mucosal immune response.
Until a few years ago, little was known about the genetic regulation of immune cell levels. In 2013, we showed the genetic contributions to the quantitative levels of 95 cell types encompassing the innate and adaptive immunity in a cohort of 2,870 SardiNIA individuals (Orrù V et al., Cell 2013). We first established that the immune variation is genetically determined, then we demonstrated the connection between immune-related disease risk alleles and levels of particular immune cell types via genetic associations. Such overlapping associations identified specific immunological populations that are unbalanced in disease status and suggested mechanisms by which specific risk alleles might lead to disease susceptibility.

To detect new loci involved in the regulation of immune cell traits and to confirm and refine the previous association signals, we expanded the previously assessed cell populations and increased the number of analyzed volunteers. In details, we designed new multicolor panels to characterize immunologic traits belonging to the innate immunity, such as monocyte subtypes and myeloid-derived suppressor cells (MDSCs), known to be related to cancer conditions and immune-diseases. The adaptive immunity has been evaluated through the assessment of T cells maturation stages, specific helper T cell subsets (including Th1, Th2, Th9 and Th17), regulatory T cells (divided in resting, activated and cytokine-secreting), follicular helper-like T cells (Tfh), and lymphoid tissue inducer cells (LTI). Moreover, we assessed the B cell profile dissecting naïve, memory, transitional and plasmablast/plasma cell subsets including gut homing markers. Overall, the current immunophenotypic dataset recapitulates the phenotypic variation of 345 actual counted cells, 597 percentages with respect to hierarchical cell lineages, 3,057 median fluorescence intensities assessed from the peripheral blood of 4,000 general population volunteers within two hours from the withdrawal. For each trait, we firstly evaluate the phenotypic variation due to the genetic component, the so-called heritability, then we perform a whole genome sequencing-based association analysis: using our Sardinian specific reference panel generated by whole sequencing data of 3,500 individuals combined with extensive genotyping of the whole Sardinia cohort and powerful imputation methods, we are able to interrogate over 26 million variants (23M single nucleotide polymorphisms and 3M insertions/deletions). Finally, we search for coincident genetic associations between immune traits and disease using Sardinian case/control studies performed in our Institute and public repositories such as GWAS Catalog and Immunobase.

In conclusion, the key points of this study approach are: a) the deep immune system characterization, aa) the dense Sardinian genetic map and aaa) the systematic search for genetic variants affecting both disease risk and immune cell levels. By this approach, we are trying to provide a better understanding of the immunological effect of genetic variants to dissect disease mechanisms and identify specific targets for interventions in personalized therapies.
The liver-specific natural killer (NK) cell population is critical for local innate immune responses, but the mechanisms that lead to their selective homing and the definition of their functionally relevance remain enigmatic.

OBJECTIVES: We took advantage of the availability of healthy human liver to rigorously define the mechanisms regulating the homing of NK cells to liver and the repertoire of receptors that distinguish liver-resident NK (lr-NK) cells from circulating counterparts.

FINDINGS: Nearly 50% of the entire liver NK cell population is composed of functionally relevant CD56(bright) lr-NK cells that localize within hepatic sinusoids. CD56(bright) lr-NK cells express CD69, CCR5 and CXCR6 and this unique repertoire of chemokine receptors is functionally critical as it determines selective migration in response to the chemotactic stimuli exerted by CCL3, CCL5 and CXCL16. Here, we also show that hepatic sinusoids express CCL3(pos) Kupffer cells, CXCL16(pos) endothelial cells and CCL5(pos) T and NK lymphocytes. The selective presence of these chemokines in sinusoidal spaces creates a unique tissue niche for lr-NK cells that constitutively express CCR5 and CXCR6. CD56(bright) lr-NK cells co-exist with CD56(dim) conventional NK (c-NK) cells that are, interestingly, transcriptionally and phenotypically similar to their peripheral circulating counterparts. Indeed, CD56(dim) c-NK cells lack expression of CD69, CCR5, and CXCR6 but express selectins, integrins and CX3CR1.

CONCLUSION: Our findings disclosing the phenotypic and functional differences between lr-Nk cells and c-NK cells are critical to distinguish liver-specific innate immune responses. Hence, any therapeutic attempts at modifying the large population of CD56(bright) lr-NK cells will require modification of hepatic CCR5 and CXCR6.

BEST POSTER PRESENTATION

Fatimata Thiombiano

ASSESSMENT OF ANTIBODIES RESPONSE AGAINST MALARIA INFECTION AFTER TREATMENT WITH ACT IN CHILDREN AND ADULTS LIVING IN MALARIA HYPERENDEMIC AREA OF BURKINA FASO

Centre National de Recherche et de Formation sur le Paludisme (Burkina Faso), Université Polytechnique de Bobo Dioulasso

Background
Artemisinin-based Combination Therapies (ACTs) are the first line drug for the treatment of uncomplicated malaria in most malaria endemic countries. They quickly clear the parasitaemia and reduce fever. In animal model, it has been found that artemisinin derivatives have an immunosuppressive effect. In the present study we assessed the effect of ACTs on malaria antigens specific antibodies production in a population living in malaria hyperendemic area.

Methods
In 2012, patients aged over 6 months and adults, presenting uncomplicated malaria were
recruited and allocated to receive ACTs and follow up to 2 years. Antibodies titers against three *P. falciparum* blood stage antigens (MSP3, GLURP R0, and GLURP R2) were measured by ELISA before treatment and twenty eight days after treatment during the first and subsequent uncomplicated malaria episodes.

Results
In total 471 volunteers were recruited for antibody measurement. Antibody levels were always high Twenty eight days after the initiation of the treatment for all tested antigens but not significant. Further against MSP3, young subject (< 5 years old) produced more antibodies after treatment (P= 0.05). Subsequent malaria episodes seems to increase antibody level when we compare consecutive episodes for GLURP-R0 antigen (0.001< P<0.0000003)

Conclusion
In human population naturally exposed to malaria with initiated and boosted immunological responses, the Artemisinin-based Combination Therapies have no immunosuppressive effect.

Keywords: ACT, Antibodies, malaria

**POSTER PRESENTATION**
Veronica Zanon

**CURTAILED T CELL ACTIVATION CURBS EFFECTOR DIFFERENTIATION AND GENERATES NATURALLY OCCURRING CD8+ T MEMORY STEM CELLS FROM NAÏVE PRECURSORS**
Laboratory of Translational Immunology, Humanitas Clinical and Research Center

The effectiveness of adoptive T cell immunotherapy depends on many characteristics, including the capacity of the transferred T cells to mediate potent effector functions and to persist in the long-term. Human CD8+ T memory stem cells (TSCM) are the least differentiated memory T cell population in humans, and demonstrated superior reconstitution capacity and anti-tumor immune responses compared to other naïve and memory subsets, both in humanized mice and following adoptive transfer in humans. However, their paucity in the peripheral blood limits clinical applications. To this regard, the molecular signals required for their generation are still poorly understood. Here we show that curtailed T cell receptor stimulation in combination with IL-7 and IL-15 curbs effector T cell differentiation and allows the generation of naturally-occurring CD45RO–CD45RA+CCR7+CD27+CD95+ CD8+ TSCM (iTSCM) from highly purified naïve T cell precursors. Viceversa, increased signaling downstream of CD3 and CD28, as achieved by stimulation titration, or augmented costimulation via CD2 induced a progressive loss of TSCM-associated T cell markers and favoured differentiation towards the effector phenotype. The differentiated iTSCM proliferated extensively in vitro, expressed lower amounts of effector-associated genes and transcription factors compared to iTeff and underwent considerable self-renewal in response to IL-15.

According to their early-differentiated phenotype, the iTSCM preferentially produced TNF and IL-2 but little IFN-g and were characterized by a lower number of mitochondria compared to highly-activated effector T cells committed to terminal differentiation. These results indicate that limiting the expression of transcriptional regulators of effector differentiation in the early phases following TCR stimulation is key to generate iTSCM for ACT immunotherapy.
Intrauterine infection/inflammation (IUI) is characterized histologically by neutrophilic infiltration of the placenta and fetal membranes and is frequently associated with preterm labor. Mechanisms of inflammation in IUI are poorly understood. We modeled IUI in the Rhesus macaque with an intra-amniotic (IA) injection of *E. coli* lipopolisaccharyde (LPS) (O55:B5, 1mg) (n=11) or saline (controls, n=13) at 80% gestation. IL-1 signaling was blocked by recombinant human IL-1 receptor antagonist (rhIL-1ra) (Anakinra) given by maternal subcutaneous (100mg 3h prior to IA LPS) and IA (50mg 1h prior to IA LPS) routes. Fetuses were delivered surgically 16h after IA injections. At delivery, amnion was dissected free of chorion and decidua parietalis. Chorio-decidua cell suspensions were used for multi-parameter flow cytometry. Detailed inflammation assessments were performed. IA LPS increased expression of phospho-IRAK1 (p-IRAK1), a critical mediator of TLR signal transduction, and IL-8 and G-CSF mRNA (neutrophil chemoattractants) in the dissected amnion. LPS caused a massive neutrophil recruitment (mean x 10^6/g±SE: Controls 0.3±0.1 vs. LPS 9.7±2.2 p<.001) in the chorio-decidua. The recruited neutrophils expressed high levels of neutrophil Elastase and IL-8 (by IHC) and a high spontaneous production of TNFa (by intracellular flow cytometry analysis). In the amniotic fluid (AF), LPS increased prostaglandin levels (PGE2: Controls 0.8±0.1 vs. LPS 2.4±0.3 ng/ml: p<.001). rhIL1ra significantly decreased LPS-induced expression of p-IRAK1 in the dissected amnion by 6-fold (p=.003), and IL-8 and G-CSF mRNA by 4-fold and 18-fold (p=.01). In the chorio-decidua, rhIL-1ra decreased neutrophil numbers by 14- fold (p=.004) and neutrophil TNFa expression by 17-fold (p=.02). In the AF, PGE2 levels decreased non-significantly by 1.6-fold upon rhIL-1ra injection. Our data emphasize a key role for the amnion in mediating IUI, through an IL-1-dependent neutrophil recruitment and activation in the chorio-decidua. Anti-IL-1 therapy may thus be beneficial in reducing placenta inflammation during IUI.

**BEST POSTER PRESENTATION**

Bouchaour, A.

**INTEREST OF GAMMA INTERFERON IN THE DIAGNOSIS OF LATENT TUBERCULOSIS**

CHU Beni Messous, Alger, ALGERIA

Introduction
A third of the world's total population, are infected with Mycobacterium tuberculosis, the bacteria that causes tuberculosis, an infectious disease, which remains asymptomatic; “it is latent tuberculosis infection”. The advantages of intradermal reaction to tuberculin "IDR" are limited in latent tuberculosis. While new blood tests have been developed to measure the production of gamma interferon "IFNγ" by activated T lymphocytes after their exposure to M.tuberculosis-
specific antigens, this is QuantiFERON TB® test. As a result, several studies have been conducted to evaluate the different strategies available (IDR, QuantiFERON TB®) for the screening for latent tuberculosis.

Objective: Evaluate the value of the QuantiFERON test in the detection of latent tuberculosis before the initiation of an immunosuppressive treatment (anti-TNF biotherapy, corticosteroids).

Materials / Methods:
The study involved 85 patients (mean age 39 (± 15) years, sex ratio 1.5W / 1M) referred to the immunology department of the CHU Beni Messous for a QuantiFERON TB® assay based on the detection of interferon-γ by the enzyme-linked immunosorbent assay (ELISA) in order to identify responses to peptide antigens associated with Mycobacterium infection in vitro.

Results: Of the 85 patients, 19 (22.3%) had a positive QFT® test (IDR-), compared with 7 (8.2%) had a positive QFT® test and positive IDR, while only 4 (4.7%) patients had a positive IDR (QFT® -). Indeterminate results were observed in 2 (2.3%) patients. If all patients with at least one positive test (IDR and / or QFT) are considered to have latent tuberculosis, it appears that the performance of the QFT® test (30.5% vs 12.9%) is better than those of the IDR with a statistically significant difference between the two tests (P = 0.009). The agreement between these two tests is 72% with a Cohen’s κ coefficient equal to 0.24 (low).

Conclusion: This study shows that the performance of the QuantiFERON test is better than that of IDR for the detection of latent tuberculosis. This test remains useful in widely vaccinated populations as in Algeria.

POSTER PRESENTATION

B. Simovic Markovic

INTRAPERITONEAL INJECTION OF MESENCHYMAL STEM CELLS INCREASES INFILTRATION OF INFLAMMATORY DENDRITIC CELLS IN THE COLON AND AGGRAVATES DEXTRAN SODIUM SULPHATE-INDUCED COLITIS IN TH2-DOMINANT MOUSE STRAIN

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Introduction. Acute dextran sulphate sodium (DSS)-induced colitis is a well-established murine model of ulcerative colitis (UC) where innate immune cells (macrophages, dendritic cells (DCs), neutrophils and eosionophils) play important role. DSS induces colon injury and inflammation in C57BL6 mice (Th1 dominant strain). On contrary, BALBc mice (Th2 dominant animals) are relatively resistant to DSS-induced injury. Several preclinical studies suggest that, due to their immunomodulatory characteristics, mesenchymal stem cells (MSCs) could be promising therapeutic agents in the therapy of UC. Nevertheless, all of these studies used C57BL6 mice, while potential of MSCs to modulate DSS-induced colitis was not tested in BALBc animals. Since some of UC patients have dominant Th2 immune response (atopic constitution), the main aim of this study was to evaluate effects of MSCs in DSS-treated Th2 dominant strain of mice.
Methods. DSS (3%, molecular weight 40kDa) was dissolved in water and given to BALBc mice in place of normal drinking water (*ad libitum*) for 7 days. Experimental mice were treated with 1×10^6 mouse bone marrow-derived MSCs via intraperitoneal injection on days 2 and 5. Disease Activity Index (DAI: weight loss, stool consistency, visible blood in feces), was used to assess the clinical signs of colitis. The histology score of colitis was calculated as the sum of “infiltration” and “damage of epithelium” sub-scores for each mouse. The cellular make up of colon and phenotype of colon-infiltrated immune cells was determined by flow cytometry.

Results. MSCs significantly increased the damage of colon tissue in DSS-treated mice. The clinical score, body weight loss, and colon shortening were significantly higher in DSS-treated animals that received MSCs. The total number of CD45+CD11c+ inflammatory DCs, which expressed co-stimulatory molecule CD40 and produce pro-inflammatory cytokines (IL-12, TNF-α and IL-1β) were significantly higher in colons of DSS-treated mice that received MSCs. There wasn’t any significant difference in total number of IL-12, TNF-α and IL-1β-producing inflammatory F4/80+CD40+ M1 macrophages and IL-10 producing alternatively activated F4/80+CD206+ M2 macrophages between groups. Also, injection of MSCs did not affect infiltration of CD45+CD11c-Ly6G+ neutrophils and CD45+CD11c-Singlec F+ eosinophils, suggesting that DCs were the main cellular targets of MSCs.

Conclusion. Intraperitoneal injection of MSCs increases infiltration of inflammatory DCs in the colon and aggravates DSS-induced colitis in Th2-dominant mouse strain.

Keywords. DSS, colitis, MSCs, dendritic cells.