

XI NATIONAL

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SYMPOSIUM - JOINT SIICA - AINI IMMUNO-MEDIATED NEUROLOGICAL DISEASES

S1.5 EMERGING ROLES OF SPECIALIZED PRO-RESOLVING LIPID MEDIATORS IN ADAPTIVE IMMUNITY AND NEUROINFLAMMATION

Alessandro Leuti¹, Jesmond Dalli², Anders Jacobsson³,
Elisa Bisicchia⁴, Valeria Sasso⁴, Maria Teresa Viscomi⁴,
Luca Battistini⁴, Mauro Maccarrone⁵, Charles Serhan²,
Valerio Chiurchiu⁴

¹Fondazione Santa Lucia, Roma

²Harvard Medical School, Boston - USA

³University of Stockholm, Stockholm - Sweden

⁴Fondazione Santa Lucia, Roma

⁵Campus Bio-Medico University of Roma, Roma

Purpose: Resolution of inflammation is a finely regulated mechanism by which specialized pro-resolving lipid mediators (SPMs), that include resolvins and maresins, modulate phlogistic processes to avoid chronicization of inflammation and collateral tissue damage. Such molecules are mostly studied in acute inflammation and in innate immune cells, but their role in chronic inflammation and adaptive immunity is still scarce.

Results and Methods: Here we show for the first time that resolvins D1 (RvD1) and D2 (RvD2) as well as maresin 1 (MaR1) not only inhibit human CD4 and CD8 T cell activation and committed circulating T-helper (T_H) 1 and T_H17 cells, but also prevented the differentiation of naïve CD4⁺ T cells into T_H1 and T_H17 via GPR32 and ALX receptors as assessed by means of polychromatic flow cytometry, qRT-PCR and immunoblotting. These effects were corroborated in vivo in mice unable to generate these SPMs. Their potential role in impacting on chronic inflammation was also tested in an experimental model of brain remote damage, whereby we found reduced levels of RvD1 and RvD2 in CSF by ELISA and increased ALX expression by means of qRT-PCR and immunoblotting in injured animals. Also, the in vivo administration of RvD1 promoted functional recovery and neuroprotection by reducing microglia and astrocyte responses as well as inflammatory-induced cell death in distally remote brain regions, which are typically characterized by a sustained chronic inflammation in response to the primary insult.

Discussion and Conclusions: Our findings unveil hitherto unknown actions of specific SPMs and suggest that the immunoresolvent role of this novel class of endogenously produced lipid mediators might also involve the adaptive compartment of immunity and the modulation of damage-induced brain inflammation, thus identifying a potentially useful avenue for steering both peripheral and central inflammation, eventually preventing autoimmunity or neuroinflammation.

References

1. Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. *Nature* 2014; 510: 92-101.
2. Basil MC, Levy BD. Specialized pro-resolving mediators: Endogenous regulators of infection and inflammation. *Nat Rev Immunol* 2016; 16: 51-67.
3. Pauter AM, Olsson P, Asadi A et al. Elovl2 ablation demonstrates that systemic DHA is endogenously produced and is essential for lipid homeostasis in mice. *J Lipid Res* 2014; 55: 718-28.
4. HsiaoH-M, Thatcher T H, Colas RA, Serhan CN, Phipps RP, Sime PJ. Resolvin D1 reduces emphysema and chronic inflammation. *Am J Pathol* 2015; 185: 3189-201.

5. Schwanke RC, Marcon R, Bento AF, Calixto JB. EPA- and DHA-derived resolvins' actions in inflammatory bowel disease. *Eur J Pharmacol* 2016; 785: 156-64.
6. Chiurchiù V, Leuti A, Dalli J et al. Proresolving lipid mediators resolvin D1, resolvin D2, and maresin 1 are critical in modulating T cell responses. *Sci Transl Med* 2016; 8: 353ra11.

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SYMPOSIUM IMMUNOREGULATION

S2.5

AN EPIGENETIC SNAPSHOT OF IL-10 COMPETENT B CELLS

Silvia Tonon¹, Francesca Mion¹, Jun Dong², Hyun-Dong Chang², Andreas Radbruch², Nicola Tamassia³,

Marco Antonio Cassatella³, Carlo Ennio Michele Pucillo¹

¹Dipartimento Area Medica, Università degli studi di Udine, Udine

²Deutsches Rheuma-Forschungszentrum (DRFZ), Berlin - Germany

³Dipartimento di medicina sezione di patologia generale, Università di Verona, Verona

Purpose: B cells are traditionally known for their role as antibodies producers, however, in the last decades, new functions have been identified, in fact, B cells also exhibit immunosuppressive functions and act as regulatory cells. Despite the great effort of several groups, our actual insight on the origin, the phenotype and molecular signature identifying regulatory B cells is still fragmentary. Regulatory B cells mainly exert their function through the production of IL-10, but it is not clear whether the cytokine expression is an intrinsic property of the cell, or if rather B cells belonging to different subsets acquire the capability to produce IL-10 following the interaction with microenvironmental components. Our hypothesis is that only few B cells quickly respond to the stimulation through the production of IL-10 as they are in a suspended and preparatory state for its production. This condition identifies "IL-10 competent B cells" that differ from IL-10 negative B cells for a different epigenetic regulation of il10 locus.

Methods: This work focused on IL-10 competent B cells, a subset that can produce IL-10 after 5 hours of stimulation with LPS, PMA and ionomycin. Taking advantage of an IL-10 secretion assay followed by cell sorting we could isolate this functional subset from several anatomical compartments and subsequently perform molecular analysis.

Results: First, we analysed DNA methylation among the il-10 gene locus of IL-10 competent B cells using a bisulphite-based method. We found that IL-10 competent B cells are less methylated on CpG sites in some regions of the il-10 locus compared to IL-10 negative B cells. Moreover, the IL-10 competent B cells showed a different methylation and acetylation of histones in the same regions.

Discussion: Among the total B cell population, IL-10 competent B cells are a particular functional subset that can rapidly produce and release IL-10 following a microenvironmental stimulation. We found that IL-10 competent B cells can be recognized among the total population as their locus is almost completely demethylated.

Conclusion: IL-10 competent B cells are a functional immune-suppressive subset characterized by a specific epigenetic signature, but not belonging to any specific B cells subsets.

SYMPOSIUM IMMUNE CHECK POINTS AND CANCER IMMUNOTHERAPY

S3.4

ANTI-PD1 THERAPY EFFECTS ON T CELL REPERTOIRE AND FUNCTIONS IN PATIENTS WITH NSCLC CANCER: A PRELIMINARY STUDY TO IDENTIFY BIOMARKERS OF EFFICACY

Giusi Barra¹, Giuseppe Pasquale¹, Carminia Della Corte², Federica Papaccio², Fortunato Ciardiello², Floriana Morgillo², Raffaele De Palma¹

¹Clinical Immunology & Allergy, ²Oncology Section, Dept of Clinical & Exp Med, Università della Campania, Napoli

Background: Immune responses protect against tumors. Conventional chemotherapy may treat cancer but its efficacy is compromised by tumor relapse. Chemotherapy "per se" have immunostimulatory effects and sustain an antitumor T cell response. Anti-PD1 antibodies are used in clinics to boost immune responses blocking of an inhibitor receptor on T cells. We evaluated the T cell repertoire and cytokines in eight NSCLC patients who underwent anti-PD1 therapy after chemotherapy.

Methods: We used PBMC to study T cell repertoire by "Spectratyping" a PCR based technique, and production of γ -IFN, IL-2, IL-4, IL-12, IL-13 and IL-17 by Quantitative PCR. Presence of cytokine message was then confirmed measuring the protein in the sera. Each patient was studied at the end of chemotherapy and after each anti-PD1 shot.

Results: We found that chemotherapy shaped a specific T cell repertoire in these patients, expanding several T cell clonotypes that were maintained by anti-PD1 administration undergoing a long-lasting expansion. Of note, a prolonged effect in term of clinical outcome was paired by a consolidated production of IL-12 and γ -IFN.

Conclusions: These data show that chemotherapy reshapes a T cell repertoire involved in antitumor response and the functional profile of these cells marked a prolonged efficient anti-tumor T cell response. Although preliminary, these results help to understand how monitor the patients undergoing therapy with anti immune-checkpoints. This is of critical importance due to the need to identify biomarkers and monitoring tools to optimize the use of these drugs, considering the high costs of these therapies

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S3.5

IL-15, TIM-3 AND NK CELLS SUBSETS PREDICT RESPONSIVENESS TO ANTI-CTLA-4 TREATMENT IN MELANOMA PATIENTS

Rossana Tallerico¹, Costanza Maria Cristiani¹, Elina Staaf²,
Cinzia Garofalo¹, Rosa Sottile², Mariaelena Capone³,
Yago Pico de Coaña⁴, Gabriele Madonna³, Eleonora Palella¹,
Mariaelena Wolodarski⁴, Valentina Carannante², Domenico
Mallardo³, Ester Simeone³, Antonio M. Grimaldi³, Sofia
Johansson², Paolo Frumento⁵, Elio Gulletta⁶, Andrea Anichini⁷,
Francesco Colucci⁸, Gennaro Ciliberto⁹, Rolf Kiessling⁴, Klas
Kärre², Paolo A. Ascierto³,
Ennio Carbone¹

¹Tumor Immunology and Immunopathology Laboratory,
Department of Experimental and Clinical Medicine, University
"Magna Græcia" of Catanzaro, Catanzaro

²Department of Microbiology, Cell and Tumorbiology (MTC),
Karolinska Institutet, Stockholm, Sweden, Stockholm - Sweden

³Melanoma Cancer Immunotherapy and Innovative Therapy
Unit, ⁴Scientific Directorate, IRCCS Istituto Nazionale Tumori
Fondazione "G. Pascale", Napoli

⁴Department of Oncology and Pathology, ⁵Karolinska Institutet
Statistical Core Facility, Karolinska Institutet, Stockholm -
Sweden

⁶Department of Health Sciences, University "Magna Græcia" of
Catanzaro, Campus - Germaneto, Catanzaro

⁷Fondazione IRCCS Istituto Nazionale dei Tumori, Department
of Experimental Oncology and Molecular Medicine, Milano

⁸Department of Obstetrics and Gynecology, University of
Cambridge Clinical School, Cambridge - United Kingdom

Purpose: Despite the success of immune checkpoint blockade
in melanoma, the majority of patients do not respond. NK cells
recognize melanoma metastasis (1-3). We hypothesized that the
T and NK cell subset frequencies and expression levels of their
receptors may predict responses and clinical outcome of anti-
CTLA-4 treatment.

Methods: We characterized the NK and T cell phenotype, as well
as serum levels of several cytokines in 67 melanoma patients
recruited in Italy and Sweden, using samples drawn prior to and
during treatment. In a smaller cohort, we also performed in vitro
functional assays. Statistical univariate analyses were performed
using the two-tailed Student's t-test for independent groups,
ANOVA followed by Bonferroni correction or Mann Whitney test
followed by Dunn's correction. p-values < 0.05 were considered
statistically significant. To analyze data in multivariate mode,
SIMCA version 14 (MKS Data Analytics Solutions, Umeå Sweden)
was applied.

Results: Survival correlated with low expression of the inhibitory
receptor TIM-3 on circulating T and NK cells prior to and during
treatment and with the increased frequency of mature CD3⁺
CD56^{dim}CD16⁺ circulating NK cells during treatment. Survival
also correlated with low levels of IL-15 in the serum. Functional
experiments in vitro demonstrated that sustained exposure to
IL-15 enhanced the expression of PD-1 and TIM-3 on both T and
NK cells, indicating a causative link between high IL-15 levels
and enhanced expression of TIM-3 on these cells. Receptor
blockade of TIM-3 improved NK cell-mediated elimination of
melanoma metastasis cell lines in vitro.

Conclusions: These observations may lead to the development
of novel biomarkers to predict patient response to checkpoint
blockade treatment. They also suggest that induction of

additional checkpoints is a possibility that needs to be considered
when treating melanoma patients with IL-15

References

1. Lakshmikanth T et al. J Clin Invest 2009; 119: 1251.
2. Ali T et al. Nat Commun 2014; 5: 5639.
3. Sottile R et al. Eur J Immunol 2016; 46: 409.

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SYMPOSIUM - JOINT SIICA - FOCIS IMMUNODEFICIENCIES

S4.5

EVALUATION OF HEALTH-RELATED QUALITY OF LIFE USING CVID_QOL QUESTIONNAIRE: IMPACT OF CLINICAL, IMMUNOLOGICAL AND THERAPY-RELATED FACTORS ON BURDEN OF DISEASE IN COMMON VARIABLE IMMUNODEFICIENCY

Federica Pulvirenti¹, Stefano Tabolli¹, Cinzia Milito¹, Valeria Vilella², Alessandro José Bastidas Parlanti³, Isabella Quinti¹

¹Department of Molecular Medicine, Sapienza University of Roma, Roma

²Dpt of Clinical Medicine, Roma

³Hospital Universitario 12 de Octubre, Madrid - Spain

Introduction: Common Variable Immunodeficiency (CVID) is a primary antibody defect characterized by high susceptibility to sino-pulmonary infections, requiring a long-life immunoglobulin (IG) replacement therapy to reduce infective manifestations. Despite IG therapy CVID patients frequently develop chronic damage, autoimmunity, granulomatous inflammation, lymphoproliferative disorders, and malignancies, that affect Health Related Quality of Life (HRQoL). Generic instrument to assess HRQoL has been used to compare general population or chronic diseases to CVID. Recently, the first CVID-specific tool, the CVID_QoL questionnaire, has been validated with the purpose to catch the detailed impact of the disease on HRQoL. The instrument includes three dimensions: Emotional Functioning (EF), Relational Functioning (RF) and Gastrointestinal and Skin Symptoms (GSS).

Objectives: Here we use the CVID_QoL questionnaire to quantify the impact of clinical manifestations, immunological characteristics and therapy schedule on HRQoL in a large cohort of CVID adults. Results: One hundred fifty-four CVID from Referral Center for Primary Immunodeficiency of Roma completed the CVID_QoL. Immunoglobulin route of administration, therapy setting, clinical and immunological data were collected from medical files. CVID_QoL, EF and RF scales correlated with age. The duration of disease did not influence HRQoL. No difference were observed between patients receiving SCIG and IVIG; no correlation was found between IgG trough level or Ig serum level at diagnosis and CVID_QoL scores. Being female, underweight, admitted in hospital, having a previous diagnosis of cancer or chronic comorbidities, taking polymedication and having an unexplained persistent enteropathy proved to be major risk factors associated with a poor health status. In particular, females had severe scores in the emotional dimension, while relational and symptoms dimension scores were similar in men and women. The number of infection correlated with a poorer HRQoL status. The experience of pneumonia, relapsing episodes of diarrhea (> 4 for year), sinusitis and bronchitis (> 2 for year) was associated to more severe CVID_QoL scores.

Conclusions: The study provides the impact of immunological, clinical and therapy-related factors assessed by CVID_QoL on the burden of disease in adult patient with CVID. This information possibly identified new areas of therapeutic interventions to improve health status in CVID.

SYMPOSIUM MUCOSAL IMMUNOLOGY

S5.5

MFSD2A PROMOTES EPOXYGENATION OF DOCOSAHEXAENOIC ACID IN GUT ENDOTHELIUM TO GENERATE PRO-RESOLVING LIPID MEDIATORS AND DAMPEN INTESTINAL INFLAMMATION

Federica Ungaro¹, Carlotta Tacconi², Luca Massimino³, Paola Antonia Corsetto⁴, Carmen Correale⁵, Philippe Fonteyne⁵, Andrea Piontini⁵, Valeria Garzarelli⁵, Francesca Calcaterra⁶, Silvia Della Bella⁶, Antonino Spinelli⁷, Michele Carvello⁷, Angela Maria Rizzo⁴, Stefania Vetrano¹, Luciana Petti⁵, Gionata Fiorino⁵, Federica Furfaro⁵, Domenico Mavilio⁶, Krishna Rao Maddipati⁸, Alberto Malesci⁹, Silvia D'Alessio⁵, Silvio Danese¹

¹Humanitas University, Rozzano

²Vladimir-Prelog-Weg 1-5/10-ETH, Zürich - Switzerland

³School of Medicine and Surgery, University of Milano-Bicocca, Milano

⁴Department of Pharmacology and Biomolecular Science, University of Milano, Milano

⁵IBD Center, Laboratory of Gastrointestinal Immunopathology,

⁶Laboratory of Clinical and Experimental Immunology,

⁷Colon and Rectal Surgery Unit, ⁹Department of Gastroenterology, Humanitas Clinical and Research Center, Rozzano

⁸Department of Pathology, Lipidomics Core Facility, Wayne State University, Detroit - USA

Purpose: The resolution of inflammation is regulated by mediators derived from ω -3 fatty acids, such as docosahexaenoic acid (DHA), that is transported by the Major Facilitator Superfamily Domain containing 2A (MFSD2A) through the endothelium of brain, retina, and placenta^{1,2}. We wondered whether MFSD2A modulates the DHA metabolism of gut endothelial cells, eventually promoting the resolution of intestinal inflammation in Inflammatory Bowel Disease (IBD).

Methods: Lipidomic analysis³ was performed on mucosal biopsies and primary human intestinal microvascular endothelial cells (HIMEC) isolated from surgical specimens of active, resolving IBD patients and healthy subjects⁴. Healthy HIMEC were transduced with a lentivirus carrying MFSD2A overexpressing vector (MFSD2A-OE), and characterized in vitro. Adoptive transfer of human circulating endothelial progenitor cells (ECFCs), overexpressing MFSD2A, was performed in CD1 nude colitic mice, along with orally administered DHA.

Results: The lipidomic analysis revealed a reduced percentage of pro-resolving metabolites derived from Cytochrome P450 epoxygenation of DHA in the inflamed mucosa, when compared with samples from healthy and resolving. We found that reduced level of epoxygenated DHA-derivatives in active tissues correlated with lower amounts of MFSD2A compared to resolving mucosa. MFSD2A, expressed by gut endothelium, exerted pro-resolving effects in HIMEC in terms of reduced pro-inflammatory markers and anti-angiogenic functions by producing beneficial epoxygenated DHA-derivatives. Transplantation of MFSD2A-OE ECFCs in DHA-fed colitic mice ameliorated intestinal inflammation, through stimulation of epoxygenated DHA-derivative release in the inflamed mucosa. MFSD2A pro-resolving effects were completely abolished by CYP inhibitor both in vitro and in vivo, demonstrating that protective functions exerted by MFSD2A depends on epoxygenated metabolites of DHA.

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Discussion: Our study provides the first evidence of MFSD2A-dependent regulation of the pro-resolving DHA CYP450-mediated epoxygenation in the IBD-associated vasculature.

Conclusion: Our approach may help a selective cohort of non-responding patients, with the advantage of avoiding immune suppression and using natural endogenous pathways to resolve inflammation.

References

1. Wong BH et al. Mfsd2a is a transporter for the essential omega-3 fatty acid DHA in eye and important for photoreceptor cell development. *J Biol Chem* 2016) doi:10.1074/jbc.M116.721340
2. Prieto-Sánchez MT et al. Placental MFSD2a transporter is related to decreased DHA in cord blood of women with treated gestational diabetes. *Clin Nutr* 2016. doi:10.1016/j.clnu.2016.01.014
3. Maddipati KR et al. Eicosanomic profiling reveals dominance of the epoxygenase pathway in human amniotic fluid at term in spontaneous labor. *FASEB J* 2014; 28: 4835-46.
4. D'Alessio S et al. VEGF-C-dependent stimulation of lymphatic function ameliorates experimental inflammatory bowel disease. *J Clin Invest* 2014; 124: 3863-78.

WORKSHOP - JOINT SIICA-SIAAIC SEVERE ASTHMA, FROM MECHANISMS TO TREATMENT

WS1.9

INVESTIGATING WASP VENOM ALLERGY: A B CELL PROLIFERATION ASSAY

Marcello Albanesi¹, Lucia Giliberti¹, Maria Pia Rossi¹, Danilo Di Bona¹, Maria Filomena Caiaffa², Luigi Macchia¹

¹University of Bari-Aldo Moro, Bari

²University of Foggia, Foggia

Purpose: In clinical practice, the diagnosis of hymenoptera venom allergy relies on the grading of the clinical symptoms and the analysis of hymenoptera venom-specific IgE. Two different pools of IgE exist: the bound pool and circulating pool. The amount of specific IgE bound on mast cells is assessed through skin testing. The circulating pool of IgE is assessed with RAST. In grass pollen allergic patients the existence of circulating allergen-specific B cells has been demonstrated. These cells proliferate upon the cognate allergen encounter [1]. Here, we analyze the B cell proliferation in response to wasp venom and wasp-specific IgE, in wasp allergic patients.

Methods: In 4 patients with history of severe adverse reactions to wasp stings we analyzed:

- Wasp-specific IgE: bound pool of IgE was assessed by skin prick testing and intradermal testing. Circulating IgE levels were measured by RAST, in serum samples;
- B cell proliferation: blood mononuclear cells were stained with carboxyfluorescein diacetate succinimidyl ester (CFSE) and cultured in the presence of wasp venom. Proliferation of CD19⁺ and CD3⁺ cells was assessed using flow cytometry.

Results: Consistently with their clinical status, all the patients had high levels of both bound and circulating wasp-specific IgE. In 3 out of 4 patients CD 19⁺ cells proliferation in the presence of the wasp venom was higher compared to the control. In contrast, CD3⁺ cells did not show a higher proliferation rate when exposed to the wasp-venom.

Discussion: We show that the patients with wasp-venom allergy have a population of circulating wasp venom-specific CD19⁺ cells.

Conclusions: These latter cells can be detected using flow cytometry and their proliferation in response to the cognate allergen can be analyzed using CFSE dye.

Reference

1. Wong KJ, Timbrell V, Upham JV et al. IgE⁺ B cells are scarce, but allergen-specific B cells with a memory phenotype circulate in patients with allergic rhinitis. *Allergy* 2015; 70: 420-8.

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WORKSHOP AUTOIMMUNE MECHANISMS AND DISEASES

WS2.6

GUT INFLAMMATION AND CUTANEOUS BARRIER LEAKAGE DRIVE SKIN AUTOIMMUNITY IN OMENN SYNDROME

Rosita Rigoni¹, Elena Fontana¹, Veronica Marrella², Virginia Maina², Anna Villa³, Barbara Cassani¹

¹Humanitas Clinical and Research Center, Rozzano

²Istituto di Ricerca Genetica e Biomedica, Milano Unit, CNR, Milano

³TIGET, Division of Regenerative Medicine, Stem Cells and Gene Therapy, IRCCS San Raffaele Scientific Institute, Milano

Purpose: In addition to inflammatory bowel disease, skin degeneration represents the hallmark of Omenn Syndrome (OS), a peculiar immunodeficiency caused by hypomorphic mutations in RAG genes leading to the generation of oligoclonal activated T cells. However, the pathogenesis still remains elusive.

Methods: Skin cell suspensions were analyzed by flow cytometry. Gene expression profiling was carried out by qPCR on total skin tissue. Colitis was induced by chronic Dextran sulfate sodium (DSS) administration in drinking water.

Results: The skin compartment of Rag2^{R229Q/R229Q} mice, the OS murine counterpart, was characterized by the lack of epidermal $\gamma\delta$ T cells and marked accumulation of dermal CD4⁺/CD8⁺ T cells producing high levels IFN γ . Increased production of T cell recruiting chemokines by keratinocytes sustained the cutaneous lymphocytic infiltration in OS mice. In agreement, peripheral CD4⁺ T cells showed higher expression of skin homing receptors. Interestingly, upregulation of Toll-Like receptors and high level of the Cramp antimicrobial peptide correlated with increased bacterial load in the Rag2^{R229Q/R229Q} cutaneous tissue. Of note, in mutant but not in wild-type mice, chronic DSS treatment amplified keratinocytes activation, upregulation of chemokines and enhanced skin infiltration by T cells.

Discussion: We demonstrate in Rag2^{R229Q/R229Q} mice that compromised skin barrier integrity and chronic gut inflammation cooperate to induce keratinocytes activation that mediates tissue T cell infiltration. Current studies are aimed to assess whether circulating gut-derived pathogenic Th1/Th17 cells in OS [1], reprogrammed with skin tropism, contribute to local autoimmunity.

Conclusion: The gut-skin axis may play a key role in the pathogenesis of OS.

Reference

1. Rigoni R et al. JEM 2016; 213: 355-75.

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WORKSHOP CHRONIC LYMPHOCYTIC LEUKEMIA. FROM BASIC SCIENCE TO TREATMENT

WS4.9

INTEGRATED CLL SCORING SYSTEM HAD PROGNOSTIC ACTIVITY ON YOUNG AND EARLY-STAGE CHRONIC LYMPHOCYTIC LEUKEMIA

Andrea Visentin¹, Federica Frezzato¹, Silvia Imbergamo¹, Valentina Trimarco¹, Veronica Martini¹, Filippo Severin¹, Flavia Raggi¹, Laura Bonaldi¹, Edoardo Scomazzon¹, Leonardo Martinello¹, Francesco Piazza¹, Monica Facco¹, Gianpietro Semenzato¹, Livio Trentin¹

¹Hematology and Clinical Immunology unit, Department of Medicine, University of Padova, Padova

Purpose: Chronic Lymphocytic Leukemia (CLL) is characterized by heterogeneous clinical course. To improve the predictive accuracy of clinical, biological and molecular markers, they have been combined into indexes. Recently, our group had identify the Integrated CLL Scoring System (ICSS) based on cytogenetic abnormalities by FISH, IGHV mutational status and CD38 expression from 212 patients [1]. The aim of this study was to validate the prognostic power of our index into a larger series of 420 CLL patients, including Binet A and younger (< 65 years) patients.

Methods: Among the 816 patients followed at the Hematology Unit of Padova University Hospital from 1989 to 2015, 420 had available data of FISH, IGHV mutation and CD38 and were recruited in this study. According to ICSS, patients were classified as: low-risk, those patients with 13q deletion or normal FISH, IGHV mutated and CD38 < 30%; high-risk, subjects with 17p or 11q deletion and/or IGHV unmutated and CD38>30%; intermediate-risk, all remaining patients.

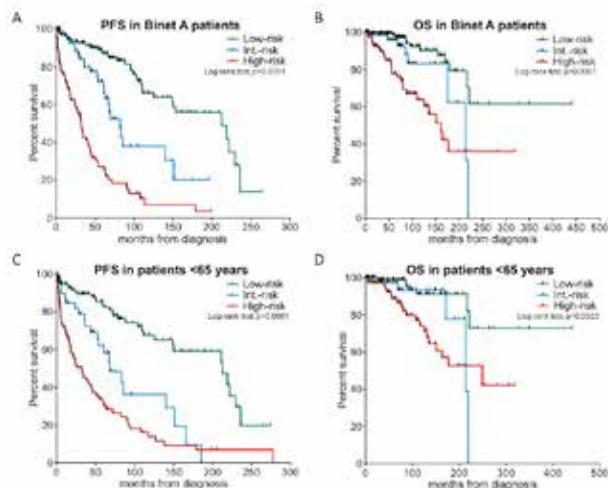
Results: The median age of our cohort was 62 years; 64% were males and 85% were Binet stage A at diagnosis. According to ICSS 202 (48%) subjects were classified as low-risk, 83 (20%) intermediate-risk and 135 (32%) high-risk. After a median follow-up of 81 months, our scoring system stratified the whole population into 3 different groups for PFS ($p < 0.0001$) and OS ($p < 0.0001$). Our score maintains its prognostic activity when considering young and Binet A patients. Among Binet A patients (85%, Figure 1A-B) the estimated 10-year PFS and OS were 66%, 38%, 6% ($p < 0.0001$) and 90%, 83%, 63% ($p < 0.0001$) for low, intermediate and high-risk groups, respectively. Considering patients < 65 years (57%, Figure 1C-D) the estimated 10-year PFS and OS were 67%, 36%, 16% ($p < 0.0001$) and 91%, 93%, 71% ($p = 0.0023$) for low, intermediate and high-risk groups, respectively.

Discussion and Conclusions: We herein provide evidence of the prognostic power and feasibility of ICSS into a cohort of 420 CLL patients and among young and Binet A patients.

References

1. Visentin A et al. Integrated CLL Scoring System, a New and Simple Index to Predict Time to Treatment and Overall Survival in Patients With Chronic Lymphocytic Leukemia. Clin Lymphoma Myeloma Leuk 2015; 15: 612-20.e1-5. doi: 10.1016/j.clml.2015.06.001.

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WORKSHOP 1 AUTOIMMUNITY

W1.1

T-CELL PROAPOPTOTIC AND ANTIFIBROTIC ACTIVITY AGAINST AUTOLOGOUS SKIN FIBROBLASTS IN VITRO IS ASSOCIATED WITH IL-17A AXIS MODULATION IN SYSTEMIC SCLEROSIS

Serena Vettori¹, Giusi Barra², Giuseppe Pasquale², Luciana Pellecchia¹, Lucia Vicedomini¹, Raffaele De Palma³

¹Rheumatology Unit, ²Allergy and Clinical Immunology Unit, Department of Internal and Experimental Medicine "F. Magrassi-A. Lanzara", University of Campania "Luigi Vanvitelli", Napoli

³Clinical Immunology & Allergy, Dept of Clinical & Exp Med, Università della Campania, Napoli

Background: IL-17A has been implicated in the pathogenesis of systemic sclerosis (SSc). We previously showed that skewed peripheral blood mononuclear cells (PBMCs) from SSc patients can induce Fas-mediated apoptosis in co-cultured autologous skin fibroblasts. We therefore aimed to investigate IL-17A expression and effects in these co-cultures.

Methods: PBMCs and skin fibroblasts from 5 dcSSc patients with disease duration < 3 years were co-cultured up to 10 days in presence of hrIL-2 [20 U/ml] in a 1:10 ratio, as previously described. IL17A, IL17RA, CXCL11, CCL2, CCL3, TGFBR2, SMAD3, CTGF, COL1A1, COL3A1 mRNA expression was assessed by Sybr Green real-time PCR. Chemokine production was further investigated at the protein level by multiple suspension immunoassay, while total collagen content was investigated by Sircol assay in culture supernatants. In subset experiments, co-cultures were treated with either IL-17A or IL-17A plus anti-IL17 receptor A monoclonal antibodies (anti-IL-17RA mAb), then cells were stained with Annexin V and anti-FAS antibodies and were investigated by flow-citometry.

Results: IL17A mRNA in co-cultured PBMCs was increased by 11.5 fold ($p < 0.01$), and IL17RA by 4.3 fold ($p < 0.05$) in co-cultured fibroblasts. CXCL11, CCL2, and CCL3 were also up-regulated at both mRNA (11.9 fold, 773.3 fold, and 29 fold, respectively; $p < 0.05$) and protein level (8.9 fold, 11.2 fold, and 252.4 fold, respectively; $p < 0.05$). Profibrotic mediators, such as COL1A1, COL3A1, and CTGF mRNA expression in co-cultured fibroblasts was reduced to 0.33 fold, 0.24 fold, and 0.31 fold, respectively ($p < 0.05$). These effects were associated with mRNA down-regulation of two key effectors of TGF- β signaling, TGFBR2 and SMAD3 to 0.59 and 0.79 fold, respectively. At flow cytometry analysis, we observed a reduction in co-cultured fibroblasts apoptosis adding IL-17RA mAb to IL-17A treated cells (39% to 16.8%; $p < 0.05$), as compared to controls treated with IL-17A and isotype IgG.

Conclusion: Our results support the role of IL-17A in the pathogenesis of SSc. Furthermore, here we first show that IL-17A up-regulation in co-cultured PBMCs might partly mediate antifibrotic effects in autologous skin fibroblasts, and might be implicated in fibroblasts apoptosis.

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W1.2

CD4⁺ MEMORY-STEM T CELLS: NOVEL PLAYERS IN RHEUMATOID ARTHRITIS

Beatrice Claudia Cianciotti¹, Eliana Ruggiero¹, Corrado Campochiaro², Giacomo Oliveira¹, Mattia Baldini², Matteo Doglio³, Angelo Andrea Manfredi⁴, Elena Baldissera², Fabio Ciceri⁵, Nicoletta Cieri¹, Chiara Bonini¹

¹Experimental Hematology Unit, Division of Immunology, Transplantation and Infectious diseases, ²Department of Internal Medicine, ³Immunotherapy Unit, Division of Immunology, Transplantation and Infectious diseases, ⁴Autoimmunity & Vascular Inflammation Unit, Division of Immunology, Transplantation and Infectious diseases, ⁵Hematology and Bone Marrow Transplantation Unit, Division of Regenerative Medicine, Stem Cells and Gene therapy, San Raffaele Scientific Institute, Milano

Purpose: Memory Stem T cells (T_{SCM}) are long living self-renewing T cells, which play a relevant role in immunological memory [1-4]. The aim of this work is to investigate the potential role of T_{SCM} as a reservoir of arthritogenic T cells in Rheumatoid Arthritis (RA).

Methods: We analysed circulating T_{SCM} (CD45RA⁺CD62L⁺CD95⁺ T cells) by flow cytometry in 27 RA patients. Fourteen patients were longitudinally followed during treatment with the anti-TNF α agent, Etanercept. We detected citrullinated antigen specific T cells using custom MHC Class II Tetramers. Cytokine production was tested with PMA/Ionomycin assay. Thirty-eight age-matched healthy donors were used as control. We high-throughput sequenced the T-cell receptor (TCR) repertoire using unbiased RNA-based approach [5] in FACS-sorted CD4⁺ T cell subpopulations from three patients.

Results: CD4⁺ T_{SCM} were significantly expanded in RA patients compared to matched controls in terms of frequency and of absolute counts. Their size contracted upon anti-TNF α treatment. Expanded CD4⁺ T_{SCM} displayed a prevalent T_H17 phenotype. Peripheral CD4⁺ T cells specific for a vimentine-derived citrullinated peptide [6], were traced, detected also in the T_{SCM} compartment, and contracted during anti-TNF α treatment. TCR-sequencing reveal a skewed RA T_{SCM} TCR repertoire, with the 10 most frequent clones accounting for 45.6% (41,3-53,7%) of T_{SCM} clones.

Discussion: The features we describe are compatible with a casual - and not only epiphenomenic - role of T_{SCM} in RA natural history.

Conclusion: The analysis of T_{SCM} dynamics in autoimmune disorders could have implications for innovative therapeutic strategies.

References

1. Gattinoni et al. Nat Med 2011.
2. Cieri et al. Blood 2015.
3. Oliveira et al. STM 2015.
4. Fuertes-Marraco et al. STM 2015.
5. Ruggiero et al. Nat Commun 2015.
6. Hill et al. J Immunol 2001.

W1.3

DEVELOPMENT OF A NOVEL EPITOPE-BASED DIAGNOSTIC ASSAY FOR SYSTEMIC SCLEROSIS

Gianluca Moroncini¹, Matteo Mozzicafreddo², Massimiliano Cuccioloni², Antonella Grieco¹, Chiara Paolini¹, Cecilia Tonnini¹, Enrico Avvedimento³, Sara Salvucci¹, Ada Funaro⁴, Armando Gabrielli¹

¹Clinica Medica, Università Politecnica Marche, Ancona

²School of Biosciences and Veterinary Medicine, University of Camerino, Camerino

³Department of Molecular Medicine, Università Federico II, Napoli

⁴Department of Medical Sciences, University of Torino, Torino

Purpose: To develop an assay for classification of systemic sclerosis (SSc) clinical subsets, based on the epitopes recognized by different autoantibodies.

Methods: a) The PDGF receptor alpha (PDGFR α) peptide library used for epitope mapping of monoclonal anti-PDGFR α antibodies [1], and two smaller libraries containing: b) the top 20 PDGFR α conformational binders and 60 conformational and linear peptides of a cognate protein forming a complex with PDGFR α ; c) the top cognate protein peptide binders, and 15 chimeric PDGFR α /cognate protein peptides, chosen among the best binders; were screened with 25 SSc (12 limited, 13 diffuse) and 25 healthy control (HC) serum samples. Libraries were synthesized by Pepscan Presto, Netherlands. Statistical analysis was performed by Wilcoxon-Mann-Whitney test. Serological and clinical data were correlated.

Results / Discussion: An immunodominant peptide discriminating SSc from HC serum samples was identified in library a). This was confirmed by library b), which highlighted also one immunodominant epitope from the cognate protein. Two cohorts of SSc samples (reactive vs nonreactive), each composed of limited and diffuse SSc subsets, were identified. Library c) identified the chimeric epitope bound exclusively by the reactive SSc serum samples, which were taken from patients with active, progressive disease regardless of limited vs diffuse subsets, whereas the nonreactive SSc samples were taken from subjects with less active, non progressive disease.

Conclusions: We developed a conformational epitope-based assay detecting SSc-specific, agonistic autoantibodies. This novel array may identify SSc patients with active disease, regardless of the canonical classification. We propose this assay for prospective screening of large cohorts of patients affected by, or suspected for, SSc, to validate it as a tool for disease activity assessment and/or early diagnosis.

Reference

1. Moroncini G et al. Arthritis and Rheumatology 2015; 67: 1891-1903.

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W1.4

IL-17 POLARIZATION OF MUCOSAL INVARIANT T CELLS DERIVES FROM THE ACTIVATION OF TWO DIFFERENT PATHWAYS

Diana Di Liberto¹, Giuliana Guggino², Francesco Ciccio², Marianna Lo Pizzo¹, Guido Sireci¹, Francesco Dieli¹, Laura Saieva¹, Riccardo Alessandro¹, Giovanni Triolo²

¹Dipartimento di Biopatologia e Biotecnologie Mediche,

²Dipartimento Biomedico di Medicina Interna e Specialistica, Università di Palermo, Palermo

Purpose: 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 [1, 2]. Recently, MAIT cells have been implicated in the pathogenesis of autoimmune disorders and found expanded in salivary glands of pSS patients [3]. Mucosal-associated invariant T (MAIT) cells recognize antigens shared by many microbes and presented by the MHC class I-like molecule MR1. Thus, their activation could determine activation of effector mechanisms. 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 cytometry 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- γ t, respectively.

Discussion: The identification of the cellular sources and inducers of IL-17 is crucial in the understanding of the drivers of inflammation in pSS.

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.

References

1. Ciccio F et al. Clin Exp Immunol 2015; 181: 219-29.
2. Lavoie TN et al. Genom Data 2015; 7: 178-84.
3. Wang JJ et al. 2016; 46: 2444-53.

W1.5

PLANT-BASED VIRUS-DERIVED NANOPARTICLES (VNPS) DISPLAYING LIPOCALIN TO USE AS COATING REAGENT IN ELISA ASSAY FOR THE DIAGNOSIS OF SJOGREN'S SYNDROME

Elisa Tinazzi¹, Ruggero Beri¹, Linda Avesani², Elena Bartoloni³, Antonio Puccetti⁴, Claudio Lunardi¹

¹Department of Medicine, ²Department of Biotechnology, University of Verona, Verona

³Department of Medicine, University of Perugia, Perugia

⁴Immunology Unit, Bambin Gesù Institute Hospital, Roma

Primary Sjogren's syndrome (SjS) is a systemic autoimmune disease characterized by complex and heterogeneous clinical features. The diagnosis may be underestimated due to the lack of sensitive and specific markers, since the presence of ANA or anti-Ro-SSA and/or anti-La-SSB antibodies can be undetectable in a number of subjects. In these setting identification of novel autoantigens may be useful in the diagnostic process

The great expansion of proteomic technologies led to uncovering specific immunodominant epitopes associated to autoimmune diseases, useful for diagnostic tools. The most widely used immunoassays are based on ELISA techniques. It was recently demonstrated that the use of plant systems as bioreactor for the production of plant-based virus-derived nanoparticles (VNPs) as scaffolds for peptide display, log increased their ability to detect specific-antibodies in sera when used as coating reagent in ELISA assay.

We exploited plant-produced Potato virus X (PVX) chimeric virus particles for displaying a peptide (lipo), which was previously described as an immunodominant peptide in Sjögren's syndrome (SjS), being recognized by patients' serum autoantibodies, and used it as building blocks for a diagnostic assay.

VNPs displaying lipo peptide were employed to set-up a direct ELISA test. In particular, PVX VNPs-lipo gave higher sensitivity and similar specificity in identifying SjS patients compared to the same test performed with the chemically synthesized immunodominant peptide.

This novel biomarker can help us in the diagnosis of patients affected by SjS and confirm the utility of plant viral nanoparticles as diagnostic devices, suggesting the possible application of this technology to the diagnosis of other autoimmune diseases.

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W1.6

TARGETED NANOPARTICLES-BASED DIAGNOSIS AND TREATMENT OF RHEUMATOID ARTHRITIS

Federico Colombo¹, Luis Nunez², Paolo Durigutto¹, Stefania Biffi³, Enrico Rampazzo⁴, Beatrice Belmonte⁵, Daniele Sblattero¹, Alessandro Gulino⁵, Pier Luigi Meroni⁶, Francesco Tedesco⁶, Paolo Macor¹

¹Department of Life Sciences, University of Trieste, Trieste

²LNK Chemsolutions LLC, Lincoln - USA

³Institute for Maternal and Child Health, IRCCS "Burlo Garofolo", Trieste

⁴Department of Chemistry "G. Ciamician", University of Bologna, Bologna

⁵Tumor Immunology Unit, Department of Health Science, Human Pathology Section, University of Palermo, Palermo

⁶Division of Rheumatology, Department of Clinical Sciences and Community Health, University of Milano Istituto Auxologico Italiano, Milano

Purpose: Disease-Modifying Antirheumatic Drugs (DMARDs) remain the main desired strategy for the treatment of RA, and Methotrexate (MTX) is still the "anchor" drug. However, many patients treated for long term showed several side effects. The aim of this work is to develop nanotechnology-based approaches for RA diagnosis and therapy able to specifically target inflamed synovial tissue in order to enhance the sensitivity and the efficacy and to reduce off-target effects.

Methods: We used targeted polymeric biodegradable nanoparticles (tBNPs), made of polylactic acid, polycaprolactone and polyethylene glycol and coated with a peptide characterized for its ability to target only inflamed synovial tissue. Biocompatibility, physical properties and inflamed synovial specificity of these tBNPs were characterized in-vitro. Afterward, the biodistribution and the efficacy of the tBNPs loaded with MTX were studied in a rat model of antigen-induced arthritis (AIA). Finally, tBNPs efficacy was also studied in a mouse model of collagen-induced arthritis (CIA).

Results: Study of in vivo biodistribution in AIA model proved the specific binding of the tBNPs for the inflamed synovial tissue with an increased concentration of tBNPs into the inflamed joints. In the same animal model, a single injection of targeted BNP loaded with MTX was enough to abrogate the inflammatory process compared with the same dose of MTX. Similar therapeutic effects were obtained in a model of CIA while no toxic effects were reported when MTX was loaded in targeted nanoparticles in these animals.

Discussion: Our results highlighted that targeted BNP were able to efficiently and selectively deliver MTX to inflamed synovial tissue of RA animal models reducing inflammation without side effects.

Conclusions: This adaptable technology could provide a new system to drive in a specific manner different molecules for an early diagnosis and for a powerful treatment of RA with minimal side effects.

W1.7

IL-21 PROMOTES GRANZYME B-DEPENDENT NK/ PLASMACYTOID DENDRITIC CELL FUNCTIONAL INTERACTION IN CUTANEOUS LUPUS ERYTHEMATOSUS

Valentina Salvi¹, William Vermi¹, Andrea Cavani², Silvia Lonardi¹, Teresa Carbone³, Fabio Facchetti¹, Daniela Bosisio¹, Silvano Sozzani¹

¹Department of Molecular and Translational Medicine, University of Brescia, Brescia

²National Institute for Health, Migration and Poverty, Roma

³Istituto Dermatologico dell'Immacolata, Roma

Purpose: Autoimmune skin lesions are characterized by a complex cytokine milieu and by the accumulation of plasmacytoid dendritic cells (pDCs)¹. Granzyme B (GrB) transcript is abundant in activated pDCs^{2,3}, though its mechanisms of regulation and biological role are largely unknown.

Methods: Freshly purified pDCs were stimulated with IL-21 for 24 hrs and GrB production was evaluated by ELISA. The expression of IL-21, MxA and GrB in lupus erythematosus (LE) skin lesions was analysed by Real-time PCR and/or immunohistochemistry. Keratinocyte apoptosis was evaluated by FACS.

Results: Here we report that IL-21 was the only Th1/Th17 cytokine able to induce the expression and secretion of GrB by pDCs and that this action was counteracted by the autocrine production of type I interferons (IFNs). In LE skin lesions, the percentage of GrB⁺ pDCs directly correlated with the IL-21/MxA ratio, indicating that the interplay between these two cytokines finely tune the levels of pDC-dependent GrB also in vivo. In LE, pDCs colocalized with professional cytotoxic cells at sites of epithelial damage, suggesting a role in keratinocyte killing. In accordance, we demonstrate that supernatants of IL-21-activated pDCs promoted autologous keratinocyte killing by NK cells and this action was dependent on GrB.

Discussion: These results propose a new GrB-dependent functional interaction between pDCs and NK cells and highlight a negative feedback regulation by type I IFNs in vitro and in vivo that may function to limit excessive tissue damage.

Conclusions: This study extends our understanding on the regulation and function of GrB production by pDCs and highlights new roles for infiltrating pDCs in skin LE lesions.

References

1. Swiecki M, Colonna M. Nat Rev Immunol 2015; 15: 471-85.
2. Rissoan MC et al. Blood 2002; 100: 3295-303.
3. Bratke K et al. Clin Exp Allergy 2010; 40: 1015-24.

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W1.8

MIR 106A, MIR 19A-B, MIR 20A AND MIR21A REGULATE V γ 9V δ 2 FUNCTIONS PARTICIPATING IN THE INFLAMMATORY RESPONSES OCCURRING IN RHEUMATOID ARTHRITIS

Giuliana Guggino¹, Valentina Orlando¹, Laura Saieva¹, Francesco Ciccia¹, Irmina Chalcarz¹, Riccardo Alessandro¹, Francesco Dieli¹, Nadia Caccamo¹, Giovanni Triolo¹

¹DIBIMIS, Palermo

Purpose: In this study we aimed to evaluate miR17-92 expression and functions of gd T cell subsets in Rheumatoid Arthritis (RA).

Methods: Polyclonal V γ 9V δ 2 T cell lines were generated by magnetic isolation followed by sorting and further analysed by flow cytometry. Expression analysis of miRNA17-92 cluster was performed by RT-PCR and mRNA expression of miRNA target genes was studied.

Results: A remarkable change in the distribution of V γ 9V δ 2 T cell functional subsets was observed in the peripheral blood of RA patients, as compared to healthy donors (HD). The comparative analysis of miRNA expression among V γ 9V δ 2 T cell subsets, between RA patients and HD showed a downregulation of miR106a-5p and miR20a-5p and an upregulation of miR 21a-5p among V γ 9V δ 2 T_{EM} cells; a downregulation of miR19-3p among V γ 9V δ 2 T_{EM} and T_C^{CM (central memory)} cells was also found. These miRNA expression regulated IL-8, IL-2 and IL-6 gene transcription contributing to the survival of the pro-inflammatory pool reducing the expression of the regulates programmed cell death 4 (PDCD4) gene.

Discussion: A downregulation of miR 106a, miR19a-b and miR 20a were detected. These three miRNA showed capability in maintenance and regulation of anti-inflammatory state. In vitro studies have demonstrated that IL-8 is a direct target of miR-106a and that miR19a-b and miR 20a regulate the IL-2, IL-6 and IL-8 genes. miRNA inhibition increased the activity of these cytokines. The down regulation of miRNA 106a, 20a and 19 was accompanied with the up regulation of miRNA 21a in RA patients. It can exert cellular proliferative effect on T cells by negatively regulates PDCD4 expression and inhibiting suppressive functions.

Conclusions: Our results provide evidence of a role of miR106a, miR19-3p, 20a and 21a in the regulation of V γ 9V δ 2 T cells function in RA patients and suggest the possibility that the miR17-92 family and gd T cells participate to the pathogenesis of RA.

References

- Philippe L, Alsaleh G, Bahram S, Pfeiffer S, Georgel P. The miR-17~92 Cluster: A key player in the control of inflammation during rheumatoid arthritis. *Front Immunol* 2013; 4: 70.
- Mogilyansky E, Rigoutsos I. The miR-17/92 cluster: a comprehensive update on its genomics, genetics, functions and increasingly important and numerous roles in health and disease. *Cell Death Differ* 2013; 20: 1603-14.

WORKSHOP 2

INNATE IMMUNITY AND INFLAMMATION I

W2.1

THE FIBRINOLYTIC AND INNATE IMMUNE SYSTEMS COOPERATE TO CONTROL AND ELIMINATE MICROBIAL SKIN INFECTIONS

William Santus¹, Francesca Mingozzi¹, Simona Barresi¹, Achille Broggi², Ivan Orlandi¹, Giulia Stamerra¹, Marina Vai¹, Alessandra Polissi³, Ivan Zanoni², Francesca Granucci¹

¹Department of Biotechnology and Bioscience, University of Milano-Bicocca, Milano

²Harvard Medical School and Division of Gastroenterology, Boston Children's Hospital, Boston, MA - USA

³Dipartimento di Scienze Farmacologiche e Biomolecolari, Univeristà degli Studi di Milano, Milano

Purpose: The purpose of our work is to understand the mechanisms of the elimination of a pathogen during skin infections.

Methods: Taking advantage of transgenic mice models that are knockout for either NFATc2 or IFN- γ and mice in which dendritic cells can be depleted, we analyzed the inflammatory process after *Candida* (C.) *albicans* infection.

Results: We identified a molecular mechanism regulating two major phases of the innate response to *Candida albicans* skin infections, the protective containment phase (abscess), and the elimination phase. During the early containment phase, TGF- β activates fibroblasts that deposit collagen around recruited polymorphonuclear cells to prevent microbial spreading. During the elimination phase, IFN- γ , by antagonizing TGF- β , permits plasmin formation through PAI-1 downregulation. Plasmin, in turn, allows abscess capsule digestion and microbial discharge from the skin. NFATc2, by controlling innate IFN- γ production, controls the second phase of anti-microbial responses. Finally, we demonstrated that the cross talk between the fibrinolytic and the innate immune system acts not only in response to fungal infections but also to bacterial infections. IFN- γ deficient mice showed the formation of a capsulated abscess after *Staphylococcus aureus* infection that was similar to those observed in NFATc2 deficient mice infected with *C. albicans*.

Discussion: Our work shows how the activation of the plasminogen/plasmin system is regulated by the innate immune system during microbial infection and its role in microbial elimination.

Conclusions: These results shed new light on the complexity of an immune response to skin infection including the fibrinolytic system as an important player in the fight against pathogens.

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W2.2

PTX3 AND FACTOR H FUNCTIONALLY COOPERATE IN PROMOTING PHAGOCYTOSIS AND KILLING OF *ASPERGILLUS FUMIGATUS*

Raffaella Parente¹, Francesca Petroni¹, Marina Sironi¹, Sonia Valentino¹, Barbara Bottazzi¹, Alberto Mantovani², Antonio Inforzato³, Marco Gobbi⁴

¹Department of Inflammation and Immunology, Humanitas Research Hospital, Rozzano

²Humanitas Clinical and Research Center; Humanitas University, Rozzano

³Humanitas Clinical and Research Center; Department of Medical Biotechnologies and Translational Medicine, University of Milano, Rozzano

⁴Mario Negri Institute for Pharmacological Research, Milano

Purpose: *Aspergillus fumigatus* (AF) is the major etiologic agent of Invasive Aspergillosis (IA), a severe infection amongst immunocompromised individuals. A pivotal role in the host resistance to this fungal pathogen is played by polymorphonuclear neutrophils (PMNs) and complement, in a functional cooperation with the long pentraxin PTX3. This has opsonic activity, and enhances phagocytosis and killing of AF conidia by PMNs via complement pathways [1]. The aim of this study was to characterize the molecular crosstalk between PTX3 and complement in the opsono-phagocytosis of AF.

Methods: Complement activation on AF was assessed by Western Blotting. AF phagocytosis by human PMNs was analysed by Flow Cytometry. Sub/del mutants were made to define structure/function of PTX3.

Results: We found that PTX3 promotes the selective recruitment of C3b (from C3 cleavage) on the conidial wall, by exclusively targeting the alternative pathway (AP) of complement. To our surprise, factor H (main inhibitor of AP) is required for such process, thus pointing to a novel function (activating rather inhibitory) of this complement regulator when combined with PTX3. Consistent with this, in phagocytosis experiments with purified human PMNs, factor H was necessary to sustain the pro-phagocytic and pro-killing activities of PTX3. Furthermore, we made a tetrameric mutant of PTX3 (as opposed to the octameric wild type protein) with superior opsono-phagocytic properties in vitro.

Conclusions and Discussion: Here we described a cooperation between factor H and PTX3 with an unexpected functional outcome: enhanced recruitment of C3b onto AF. Given the potent opsonic activity of C3b (that is recognized by the phagocytic receptor CR1/CD35), we believe that this is the major mechanism of PTX3 in the promotion of AF phagocytosis and killing. Moreover, we have generated a new PTX3-derived protein with better activity in vitro and greater potential in vivo.

Reference

1. Cunha et al. N Engl J Med 2014; 370: 421-32.

W2.3

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

Marco Pio La Manna¹, Valentina Orlando¹, Francesco Dieli^{1,2}, Paola Di Carlo³, Antonio Cascio³, Gilda Cuzzi⁴, Fabrizio Palmieri⁴, Delia Goletti⁴, Nadia Caccamo²

¹Central Laboratory of Advanced Diagnosis and Biomedical Research (CLADIBIOR) A.O.U.P. Paolo Giaccone, Palermo

²Dipartimento di Biopatologia e Biotechnologie Mediche, Università di Palermo, Palermo

³Dipartimento di Scienze per la Promozione della Salute e Materno Infantile "G. D'Alessandro" Università di Palermo, Palermo

⁴Translational Research Unit, National Institute for Infectious Diseases L. Spallanzani, Roma

Purpose: We evaluated the ratio of monocytes to lymphocytes (ML ratio) and the ex-vivo expression of CD64 on monocytes, as biomarkers for TB stages discrimination.

Methods: A total of 173 individuals were enrolled: 31 Healthy Donors (HD); 37 latent TB infected (LTBI); 71 with active TB disease; 34 cured TB patients. Full peripheral blood counts were performed using a hematology analyzer. Monocytes were stained using mAbs to CD14, CD64, CD16, CD123, CD152, CD163, CD206, HLA-DR, CD3, CD19, CD56 and their isotype controls. Flow cytometry were performed on FACSCalibur. Median or geometric mean fluorescence was used for analysis. The Kruskal-Wallis was performed comparing the medians of ML ratio, the relationship between variables was evaluated using Spearman rank test, $p < 0.05$ was considered statistically significant.

Results: Significant differences were found comparing the average ML ratio of active TB patients with those of LTBI, cured TB and HD. The receiver operator characteristics curve analysis allowed the discrimination of active TB from HD groups, with a sensitivity of 91.04% and a specificity of 93.55% and a sensitivity of 85.07% and a specificity of 85.71% comparing TB with LTBI. Moreover, an upregulation of CD64 expression on circulating monocytes in active TB patients was found.

Discussion: Patients with active TB disease had a higher ML ratio, as compared to HD, LTBI and also cured TB patients, suggesting that the ML ratio change after anti-TB therapy and could be used to evaluate treatment success. The ML ratio increase significantly correlate with increased monocyte counts and lower lymphocyte counts. Increased expression of CD64 in TB patients might reflect differential cytokine production related to different stages of Mtb infection/disease.

Conclusion: The evaluations of ML ratio and phenotype of monocytes can be instrumental for TB stages identification.

References

1. Goletti D et al. Infect Dis Rep 2016; 24: 8 (2).
2. Yang J et al. Biomark Res.2014; 7 (2).

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W2.4

ROLE OF NEUTROPHILS IN AN IMIQUIMOD-INDUCED MOUSE MODEL OF PSORIASIS

Dalila Bevilacqua¹, Sara Costa¹, Olivia Marini¹, Elena Caveggion¹, Claire Abram², Clifford A Lowell², Giampiero Girolomoni³, Marco Antonio Cassatella¹, Patrizia Scapini¹

¹Department of Medicine, University of Verona, Verona

²Department of Laboratory Medicine, University of California, San Francisco - USA

³Department of Medicine, Dermatology and Venereology section, Verona

Purpose: Psoriasis is a chronic skin disease associated with deregulated interplays between immune cells and keratinocytes. Although a prominent skin infiltration of neutrophils is a distinctive hallmark feature of psoriatic inflammation (1), the role of neutrophils in psoriasis pathogenesis remains unclear. Aim of this study was to investigate the specific contribution of neutrophils during psoriatic inflammation.

Methods: Psoriasis was induced by topical application of AldaraTM, 5% Imiquimod (IMQ) cream (2) in B6 mice treated or not with anti-Ly6G (1A8) antibody to deplete neutrophils. Disease development was evaluated by flow cytometry and gene expression analysis of draining lymph nodes and skin biopsies, as well as by histological evaluation of skin inflammation.

Results: Neutrophil depleted mice manifested a significant increase in the recruitment of activated $\gamma\delta$ T cells in the draining lymph nodes in response to IMQ treatment. These findings, correlated with a significant increased expression of several inflammatory mediators, as well of epidermal acanthosis, in the skin of IMQ-treated neutrophil depleted mice. In line with the latter observation, we demonstrated that neutrophils inhibited $\gamma\delta$ T cell proliferation and IL-17 production in vitro and that catalase prevents this suppression.

Discussion: Overall, these data demonstrate that neutrophils may negatively contribute to disease propagation and exacerbation in the IMQ-induced mouse model of psoriasis by impairing $\gamma\delta$ T cell effector functions. We also demonstrated a potential role for neutrophil derived reactive oxygen species (ROS) in this inhibitory function.

Conclusion: Future research on the mechanisms and implications of neutrophil mediated inhibition of $\gamma\delta$ T cells is needed in order to define potential novel regulatory axis able to modulate disease pathogenesis.

References

1. Perera GK et al. Annu Rev Pathol 2012; 7: 385-422.
2. van der Fits L et al. J Immunol 2009; 182: 5836-45.

W2.5

INNATE IMMUNE ROLE OF THE LONG PENTRAXIN PTX3 IN THE CONTROL OF STREPTOCOCCUS PNEUMONIAE INVASIVE INFECTIONS

Rémi Porte¹, Raffaella Parente², Marina Sironi², Fabio Pasqualini², Maria Luisa Barbagallo², Rita Silva-Gomes², Sadaf Davoudian², Tom Van der Poll³, Cecilia Garlanda², Barbara Bottazzi², Alberto Mantovani²

¹Humanitas University, Rozzano (Milano)

²Humanitas Clinical and Research Hospital, Rozzano (Milano)

³Academic Medical Centre, Center for Experimental and Molecular Medicine and the Division of Infectious Diseases, University of Amsterdam, Amsterdam - The Netherlands

Purpose: The long pentraxin 3 (PTX3) is a fluid phase pattern recognition molecule linking the cellular and humoral arms of innate immunity. PTX3 is an important component of host resistance to pulmonary infections with selected microorganisms, including *Aspergillus fumigatus* and *Pseudomonas aeruginosa*. Our aim was to investigate the role of PTX3 in the control of pneumococcal infections caused by *Streptococcus pneumoniae*, the most common causative bacteria in community-acquired pneumonia and an important cause of mortality world-wide, especially in aged individuals.

Methods: By using a murine model of invasive pneumococcal infectious disease we analyzed the local colonization and the bacterial dissemination (bacterial count in the lung and spleen respectively). PTX3 expression during the different stages of infection was characterized by different techniques (mRNA analysis, ELISA and immunohistochemistry). Binding of PTX3 on bacteria was realized with a specific assay revealed by Western Blot.

Results: We found that PTX3 is highly expressed in situ at the local site of the infection but also in the systemic compartment following the invasive kinetic of the infection, in correlation with an important up-regulation of PTX3-inducing cytokines (e.g. TNF- α and IL-1 β). A specific binding of human and mouse PTX3 on *S. pneumoniae* was observed, independently of the capsular serotype. Comparing the pneumococcal burden in PTX3 deficient and competent mice, we observed a higher sensitivity of Ptx3^{-/-} mice during the invasive phase of the infection. Interestingly, the exogenous administration of PTX3 to Ptx3^{-/-} mice restored the sensitivity to the pneumococcal infection at the level of PTX3 competent mice. We finally showed that an exogenous local instillation of PTX3 during the ongoing infection induces an antibacterial activity on the pulmonary pneumococcal load, impacting the bacterial dissemination.

Conclusion and Discussion: Our results suggest a role of PTX3 in the control of *S. pneumoniae*. Given that pulmonary infections by *S. pneumoniae* are characterized by a consistent recruitment of neutrophils, we are currently investigating the effect of PTX3 on opsonophagocytosis of *S. pneumoniae*. This will allow to characterize a possible mechanism involved in PTX3 control of pneumococcal pulmonary infections.

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W2.6

COMPLEMENT PROTEIN C1Q PRODUCTION IN MALIGNANT PLEURAL MESOTHELIOMA

Leonardo Amadio¹, Alessandro Mangogna², Chiara Agostinis¹,
Romana Videgar², Marco Confalonieri³,
Claudio Tripodo⁴, Oriano Radillo¹, Bulla Roberta²

¹Institute for Maternal and Child Health, Istituto di Ricovero e
Cura a Carattere Scientifico Burlo Garofolo, Trieste

²Department of Life Sciences, ³Clinical Department of Medical
Sciences, Surgical and Health, University of Trieste, Trieste

⁴Department of Human Pathology, University of Palermo,
Palermo

Purpose: The complement component C1q has been shown to be abundantly expressed in the microenvironment of several solid tumors where it shows pro-tumor activities (1). We demonstrated that C1q is abundantly present in malignant pleural mesothelioma (MPM), and promote adhesion, migration and invasion of MPM tumor cells. The aim of our study was to investigate the cells and the mechanisms responsible for its local production.

Methods: MPM sections were analyzed by immunohistochemistry for the presence of C1q in the microenvironment. MPM human primary mesothelioma cells were isolated from pleural biopsy, characterized by immunofluorescence, cytofluorimetric analysis and their production of cytokines was evaluated by qPCR and ELISA. Human macrophages were incubated with MPM conditioned medium and their phenotype and their production of C1q, was evaluated by qPCR and ELISA.

Results: C1q was expressed in tumor-associated stroma of different histotypes of mesothelioma. C1q pattern distribution seems connected to tumor-infiltrating myeloid elements. MPM cells were unable to produce C1q. Mφ treated with MPM conditioned medium have shown an M2-like phenotypic profile (CD206 and IL-10 upregulation) and a significant upregulation in C1q production. No variation was detected for C1s gene.

Discussion: C1q has been shown able to induce M2-like polarization of Mφ (2). MPM cells increase the C1q production by Mφ. Higher C1q presence in the microenvironment could lead to a stronger M2-like polarization of Mφ producing a self-sustained cycle that could promote tumor malignant progression.

Conclusions: C1q fulfill a key role as an immunosuppressive and cancer-promoting factor in mesothelioma microenvironment.

References

1. Bulla R et al. Nat Commun 2016; 7: 10346.
2. Son M et al. Blood 2016; 128: 2218-28.

WORKSHOP 3

ADAPTIVE IMMUNITY

W3.1

EOMES IS EXPRESSED BY NON-CLASSIC TH1 CELLS AND IS INVOLVED IN THEIR PHENOTYPE STABILITY

Alessio Mazzoni¹, Maria Caterina Rossi¹, Manuela Capone¹,
Laura Maggi¹, Veronica Santarlasci¹, Gianni Montaini¹,
Beatrice Rossetti¹, Francesco Liotta¹, Lorenzo Cosmi¹,
Francesco Annunziato¹

¹University of Florence, Florence

Purpose: To understand why Th17-derived Th1 cells (non-classic Th1), despite exhibiting RORγt mRNA expression and IL17 locus demethylation, cannot be reverted to IL17 production

Methods: Cytokine expression was evaluated by intracellular flow cytometry staining; transcription factors were evaluated at mRNA level by Real-Time PCR; gene overexpression was achieved by lentiviral transduction.

Results: We cultured non-classic Th1 cells with different combinations of Th17-favoring cytokines, but we were never able to revert them to IL-17 production. We performed transcriptome analysis searching for genes possibly responsible for the inhibition of the IL17 phenotype, and we found high Eomes mRNA expression in non-classic Th1 cells, if compared to Th17. These data were confirmed by Real Time PCR and confocal microscopy. Eomes plays a pivotal role in NK and CD8 T cells, where it controls IFNγ expression. There is lack of information regarding its role in CD4 T cells; it was shown to inhibit RORγt and IL17 expression in murine naïve T cells during Th1 polarization. Eomes mRNA levels in T cell clones negatively correlated to RORγt mRNA expression, and its expression in Th17 cells was induced upon IL12 signaling. Moreover, Eomes forced overexpression in Th17 cells dramatically diminished IL17 production while partially increasing that of IFNγ, both at mRNA and protein level. We also observed a significant reduction of RORγt mRNA levels in Eomes transduced Th17 cells.

Discussion: Th17 cells are plastic and can modulate their phenotype towards Th1 upon appropriate cytokine instruction. This seems to be a one-way plasticity, since we were not able to revert them in vitro to IL17 production. Here we demonstrate that Eomes is expressed in non-classic Th1 cells and is responsible for the stability of their phenotype.

Conclusion: Eomes is expressed by non-classic Th1 cells and is fundamental for the inhibition of Th17 associated genes.

Reference

Yang Y et al. J Immunol 2008; 181: 8700-10.

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W3.3

HUMAN CYTOTOXIC T LYMPHOCYTES FORM DYSFUNCTIONAL IMMUNE SYNAPSES WITH B CELLS CHARACTERIZED BY NON-POLARIZED LYTIC GRANULE RELEASE

Vanessa Zurli¹, Giuliana Wimmer¹, Veronica Candi², Alessandra Gamberucci³, Alessandro Gozzetti², Giuseppe Campoccia⁴, Monica Bocchia², Cosima Tatiana Baldari¹, Anna Kabanova¹

¹Department of Life Science, University of Siena, Siena

²Hematology Unit, University of Siena, Siena

³Department of Molecular and Developmental Medicine, University of Siena, Siena

⁴Department of Immune Haematology and Transfusion Medicine, University Hospital of Siena, Siena

Purpose: Suppression of the cytotoxic T cell (CTL) immune response has been proposed as one mechanism for immune evasion in cancer. In this study, we have explored the underlying basis for CTL suppression in the context of B cell malignancies.

Methods: We used functional assays, confocal microscopy and signaling studies on human freshly isolated lymphocytes to illustrate a peculiar dysfunctional interaction between resting B cells and effector CTLs, unexpectedly shared by both malignant and healthy cells.

Results: We document that human resting B cells have an intrinsic ability to resist killing by freshly isolated CTLs, but are susceptible to lysis by in vitro expanded CTL blasts (1). Impaired killing was associated with the formation of dysfunctional immune synapses characterized by non-polarized release of lytic granules. An impaired convergence of lytic granules toward the synapse was associated with a selective blockade of the TCR signaling at the level of linker for activation of T cells (LAT). The non-polarized release of the lytic granules was achieved through Arl8-assisted granule movement toward the cell periphery.

Discussion: We found that B cells can instruct CTLs to release lytic granules in a non-polarized fashion and thus protect themselves from being killed. This may be relevant in the context of B cell malignancies. Moreover, our work underscores the importance of studying freshly isolated CTLs and the function of LAT that could provide important insights into the mechanisms regulating lytic granule transport in CTLs.

Conclusions: We propose that non-lytic degranulation of CTLs is a key regulatory mechanism of evasion through which B cells may interfere with the formation of functional immune synapses by CTLs.

Reference

1. Kabanova et al. Human CTLs form dysfunctional immune synapses with b cells characterized by non-polarized lytic granule release. Cell Reports 2016; 15: 9-18.

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W3.4

INHIBITION OF NOTCH-MEDIATED CROSSTALK BETWEEN ENDOTHELIAL CELLS AND PLASMACELLS REDUCES ANGIOGENESIS IN MULTIPLE MYELOMA PATIENTS

Ilaria Saltarella¹, Aurelia Lamanuzzi¹, Patrizia Leone¹, Vito Racanelli¹, Roberto Ria¹, Angelo Vacca¹

¹Department of Biomedical Science, Section of Internal Medicine, University of Bari "Aldo Moro" Medical School, Bari

Purpose: In Multiple Myeloma (MM) interactions between plasmacells (PCs) and endothelial cells (ECs) triggers tumor progression and stimulates bone marrow angiogenesis. Notch pathway is an high conserved signaling which regulates gene expression favoring proliferation, survival and cell differentiation. Cell-to-cell contact activates Notch signaling after the cleavage of Notch receptors by a γ -secretase [1]. We have already demonstrated that MMECs showed a high activation of Notch1 and Notch2 receptors and a strong expression of Notch target genes Hey1 and Hes1. Based on our previous results, we aim to investigate the role of Notch pathway in the crosstalk between MMECs and tumor PCs.

Methods: Real-time RT PCR and western blotting were performed to assess Notch activation in MMECs in culture with PCs line RPMI-8226. Functional in vitro assays were conducted after the treatment with siNotch1, siNotch2 and γ -secretase inhibitor on MMECs in the three experimental conditions: i) alone; ii) direct or iii) indirect culture with PCs.

Results: Co-culture conditions determined an increase of Hes1 expression, on the other hand Hey1 expression was not significantly modulated. Regarding functional assays, both Notch1 and Notch2 inhibition reduced chemotaxis (40% and 35%), adhesion (10% and 50%), spontaneous migration and in vitro angiogenesis on Matrigel[®] of MMECs. Similar data were obtained when Notch pathway was inhibited with MK-0752 with no significant differences between the three experimental conditions. Finally, MK-0752 treatment also affected RPMI-8226 survival, adhesion and gene expression.

Discussion: Notch pathway inhibition affected angiogenic capabilities of MMECs in culture with PCs.

Conclusions: BM angiogenesis and MM progression are enhanced by the existence of active interactions between PCs and MMECs. Thus, γ -secretase inhibitors could assume a central role in developing new drugs targeting Notch pathway in BM milieu.

Reference

1. Nefedova Y et al. Blood 2008.

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W3.5

DRUGS-LOADED NANOPARTICLES: A NEW APPROACH FOR THE TREATMENT OF B-CELL MALIGNANCIES

Sara Capolla¹, Riccardo Bomben², Sonia Zorzet¹, Stefania Biffi³, Gabriele Pozzato⁴, Claudio Tripodo⁵, Gustavo Larsen⁶, Luis Nùñez⁶, Paolo Macor¹

¹Department of Life Sciences, ⁴Dipartimento Universitario Clinico di Scienze mediche, Chirurgiche e della Salute, University of Trieste, Trieste

²Clinical and Experimental Onco-Hematology Unit, Centro di Riferimento Oncologico, I.R.C.C.S., Aviano (PN)

³Institute for Maternal and Child Health - IRCCS "Burlo Garofolo", Trieste

⁵Department of Human Pathology, University of Palermo, Palermo

⁶LNK Chemsolutions LLC, Lincoln (NE) - USA

Purpose: Current approaches for the treatment of chronic and aggressive B-cell malignancies have greatly improved the prognosis and survival, but some patients develop toxicity, remain refractory or are resistant to these therapeutic regimens. Currently, the major challenge is to specifically deliver the therapeutic agents to neoplastic cells while preserving the viability of healthy tissues. This aim was addressed by nanotechnology; high concentration of drugs (anti-microRNAs, fludarabine, bendamustine or the combination of hydroxychloroquine and chlorambucil) were loaded inside biodegradable nanoparticles (BNPs) conjugated with antiCD20 antibodies.

Methods: The binding (flow cytometry, confocal microscopy), internalization (TEM) and cytotoxicity (MTT, AnnexinV/PI assays) of BNPs on tumor B-cell lines and primary cells purified from patients were assessed in vitro. Then, xenograft mouse models were induced to assess the therapeutic effect of BNPs in vivo.

Results: The binding and internalization of BNPs inside tumor B-cells and their consequent cytotoxicity were proved in vitro. In vivo studies in healthy mice demonstrated the safe toxicological profile of BNPs while free drugs killed all the treated animals. The therapeutic effect of BNPs was evaluated in mouse models of chronic lymphocytic leukemia, Burkitt's lymphoma and mantle cell lymphoma; targeted BNPs cured 50-90% of treated animals while untargeted, empty BNPs and free drugs were ineffective.

Discussion: The conjugation of antiCD20 antibodies led to the specific binding of BNPs on CD20-expressing cancer cells without affecting healthy tissues; BNPs were demonstrated to affect the pharmacokinetics of drugs, resulting in the complete abolishment of the side effects and the increased efficacy of drugs.

Conclusions: Drugs-loaded antiCD20-conjugated BNPs can be effective in controlling leukemia and lymphomas providing a rationale for adopting this approach for the treatment of human CD20-expressing B-cell malignancies.

W3.6

CYTOMEGALOVIRUS CONTRIBUTES TO AGE-RELATED DYSFUNCTIONS IN THE MAINTENANCE OF IMMUNOLOGICAL MEMORY IN THE BONE MARROW

Luca Pangrazzi¹, Erin Naismith¹, Beatrix Grubeck Loebenstein¹, Klemens Trieb²

¹Institute for Biomedical Aging research, University of Innsbruck, Innsbruck - Austria

²Klinikum Wels, Wels - Austria

Purpose: Aging induces a basal level of inflammation throughout the body, a condition known as inflammaging, which contributes to immunosenescence. Alongside the extrinsic factors, Cytomegalovirus (CMV) has been considered one of the most important propagators of immunosenescence. Depending on the geographical area, 60 to 100% of the adult population are infected by the virus. CMV infection leads to a very prominent T cell response, which occupies >20% of the total CD8+ T cell pool. It has been suggested that CMV-driven memory T cell expansions significantly accelerate the age associated loss of naïve T cells, decreasing de novo immune responses.

It has been demonstrated that memory T cells home to bone marrow niches, well organized structures which promote the survival of these cells through homeostatic proliferation. T cell survival is promoted by IL-7 and IL-15. IL-7 is believed to be important for long-lived memory T cells while IL-15 is mostly important for more differentiated T cells. In addition, high IL-15 levels contribute to inflammation and tissue damage in the elderly, supporting the survival of highly differentiated T cells. In a previous study, we demonstrated that IL-15 bone marrow levels increase while IL-7 decreased in old age. Furthermore, we described how pro-inflammatory molecules and oxidative stress may play a role in the age-related dysfunction in the maintenance of immunological memory. In the current study, we describe how CMV influences the expression of T cell survival molecules, which contributes to the expansion of certain T cell pro-inflammatory subsets.

Methods: Human bone marrow samples were collected in collaboration with the Clinics of Wels-Grieskirchen. qPCR and FACS experiments are performed in order to address our questions.

Results: In our study, we obtained samples from a large group of CMV seronegative people coming from an interesting Austrian cohort in which around 40% of the donors, even in very old age, were CMV-. The expression of IL-15, and pro-inflammatory molecules IFN γ and TNF in the bone marrow was higher in CMV seropositive donors. Lower IL7R and higher IL-2/IL-15R β expression on T cells was found in CMV+ compared to CMV-donors. Age-related changes in the expression of both molecules in T cell subsets were observed. Interestingly, CMV+ donors showed different trends compared to the CMV- counterpart.

Discussion: According to our results, the maintenance of immunological memory in the bone marrow is reduced with CMV. In particular, IL-7 signaling may be impaired. In parallel, the IL-15 pathway may be potentiated in the presence of the virus, resulting in a T cell pro-inflammatory phenotype. Niches for effector/exhausted T cells in the aged BM expand in CMV seropositive individuals.

Conclusion: Our results suggest that the maintenance of immunological memory may be improved without CMV. Vaccinations against CMV should be introduced to protect from degeneration of the immune system in old age.

(524) T cell items

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W3.7

T-CELL PHENOTYPE AND FUNCTION DURING HUMAN ACUTE ZIKA VIRUS INFECTION

Eleonora Cimini¹, Concetta Castilletti², Alessandra Sacchi³, Rita Casetti³, Veronica Bordoni³, Antonella Romanelli³, Federica Turchi³, Federico Martini³, Nicola Tumino³, Emanuele Nicastrì⁴, Angela Corpolongo⁴, Antonino Di Caro², Gary Kobinger⁵, Alimuddin Zumla⁶, Giuseppe Ippolito⁷, Maria Rosaria Capobianchi², Chiara Agrati³

¹Cellular Immunology and Pharmacology Laboratory, ²Virology Laboratory, ³Cellular Immunology and Pharmacology Laboratory,

⁴Clinical Department, ⁷Scientific Direction, NMI-IRCCS "L. Spallanzani", Rome

⁵National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg - Canada

⁶Division of Infection and Immunity, University College London, London - United Kingdom

Purpose: Zika virus (ZIKV) has recently emerged as severe global health issue. Understanding host protective immunity to ZIKV may represent a key issue in vaccine efficacy definition and in identifying possible immune-pathogenetic mechanisms in severe infections. Aim of this study was to analyze the cellular immune response in the acute phase of ZIKV infection, and its role in the protection and/or pathogenesis.

Methods: T cells profile was analyzed in 7 acute ZIKV-infected patients and compared with 4 acute Dengue virus (DEGV)-infected patients and with 6 healthy donors (HD). Phenotype/Functionality of T-cells were analyzed by flow cytometry, Elispot and proliferation/degranulation assays.

Results: A significant increase in CD8 T-cells was observed in both ZIKV/DEGV patients. CD8/CD4 T-cells were activated both in ZIKV/DEGV, expressed an effector phenotype and a high level of CD95 marker. Cytokines profiling showed a lower frequency of IFN- γ -producing CD4 T-cells in ZIKV respect to DEGV. A significant expansion of T-cells expressing V δ 2 TCR was specifically observed in ZIKV but not in DEGV patients. V δ 2 T-cells from both ZIKV/DEGV patients showed a higher level of Granzyme and a lower proliferation capability than HD. A reduction of IFN- γ single positive V δ 2 T-cells was observed in ZIKV respect to both DEGV/HD. In vitro experiments showed that healthy V δ 2 T-cell release Granzyme after recognition of ZIKV-infected cells.

Discussion: Results showed that ZIKV infection induced a T-cell activation/differentiation by modulating the cytokine profile, and an expansion of cytotoxic V δ 2 T-cells, suggesting a protective role of these cells.

Conclusions: These findings provide new knowledge on the immune response profile during ZIKV infection pointing out the possible protective role of V δ 2 T-cells in controlling ZIKV replication.

References

1. Vantourout P. Nature Rev Immunol 2013.
2. Agrati C. Cell Death Dis 2016.
3. Tappe D. Med Microbiol Immunol 2015.
4. Mathew A. Immunol Rev 2008.

WORKSHOP 4

INNATE IMMUNITY AND INFLAMMATION II

W4.1

HUMAN CIRCULATING GROUP 2 INNATE LYMPHOID CELLS PROMOTE IGE PRODUCTION AND CAN BE SHIFTED IN VITRO TOWARD IFN- γ PRODUCING CELLS

Beatrice Rossetti¹, Laura Maggi¹, Gianni Montaini¹, Alessio Mazzoni¹, Manuela Capone¹, Maria Caterina Rossi¹, Veronica Santarlasci¹, Francesco Liotta^{1,2}, Oliviero Rossi^{1,2}, Enrico Maggi^{1,2}, Lorenzo Cosmi^{1,2}, Francesco Annunziato^{1,2}

¹Department of Experimental and Clinical Medicine and DENOTHE Center, University of Florence, Firenze

²Regenerative Medicine Unit and Immunology and Cellular Therapy Unit of Azienda Ospedaliera Careggi, Firenze

Purpose: Provide a comparative functional characterization between human circulating type II Innate Lymphoid cells (ILC2) and Th2 cells, and evaluate their plasticity in vitro.

Methods: Circulating ILC2 and Th2 cells isolated by MACS and FACS sorting from the same donor were expanded in vitro. Transcription factors (TF) expression levels were evaluated by Real-Time RT-PCR. Cytokines and TF gene methylation was assessed by bisulfite DNA sequencing. Flow cytometry was used to evaluate cytokines production and CD154 expression in cell lines stimulated with PMA/Ionomycin (P/I), IL-25/IL-33 or Toll-like receptor (TLR) ligands mix. IgE production by autologous B cells was assessed by ELISA. ILC2 cultured in presence of polarizing cytokines were evaluated for cytokines production following P/I stimulation.

Results: ILC2 display GATA3, RORC and RORA expression and, in response to P/I, type 2 cytokines production. Likewise, epigenetic analysis show demethylated IL4, IL13, IL5, GATA3 and RORC2 gene loci, and highly methylated IFNG and TBX21 ROIs. Besides IL-25/IL-33, also TLR stimulation induces IL-5 and IL-13 production in ILC2. In presence of the same stimuli, they express CD154 and promote IgE production. Moreover, ILC2 cultured in presence of polarizing cytokines, acquire T-bet expression and IFN- γ production. Accordingly, significant reduction of IFNG promoter methylation has been assessed.

Discussion: These data show that human ILC2 can induce IgE production independently of T cells. Moreover, ILC2 plasticity in vitro, suggest that a modulation may occur also in vivo in response to microenvironmental stimuli, and this could happen also as consequence of therapeutic interventions in type 2 associated diseases.

Conclusion: Upon IL-25/IL-33 and TLR ligands mix stimulation, human ILC2 promote IgE production in autologous B cells. In addition, ILC2 shift toward IFN- γ production under polarizing condition.

Reference

1. Maggi L et al. PMID: 27576126

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W4.2

NEUTROPHIL DYSFUNCTION IN AN INDUCIBLE MOUSE MODEL OF GLYCOGEN STORAGE DISEASE TYPE 1b

Federica Raggi¹, Anna Livia Pissavino¹, Roberta Resaz¹, Daniela Segalerba¹, Luca Mastracci², Federica Grillo², Andrea Puglisi³, Maria Carla Bosco¹, Luigi Varesio¹, Alessandra Eva¹

¹Laboratory of Molecular Biology, G. Gaslini Institute, Genova

²Department of Surgical and Diagnostic Sciences (DISC), Anatomic Pathology Unit, University of Genova, IRCCS AOU San Martino-IST, National Cancer Research Institute, Genova

³Laboratory of Molecular Biology, G. Gaslini Institute, Genova, Italy, Genova

Purpose: Neutrophils play a key role in host protection against microbial infections. Neutropenia and neutrophil dysfunction are common features of many diseases and are observed in patients affected by Glycogen Storage disease type 1b (GSD-1b)¹. GSD1b is a rare genetic autosomal recessive disease caused by the defect of the glucose-6-phosphate transporter (G6PT). The objective of this study was to understand the pathophysiology of the disease focusing on neutrophils functional activity in an inducible KO mouse model.

Methods: G6ptlox/w mice were crossed with transgenic mice expressing a TM inducible Cre-mediated recombination system². To induce the excision of G6PT exons, five-week old mice were injected intraperitoneally with TM for five days. Histological examinations of tissues and functional analysis of bone marrow/peritoneal exudate neutrophils were performed in TM-G6PT-/- mice and compared to controls.

Results: TM-G6PT-/- mice showed pathological abnormalities characteristic of the human disease, including hepatomegaly, nephromegaly and hyperlipidemia. Bone marrow and peritoneal neutrophils from G6PT-KO mice displayed impaired mobility, chemotaxis, as well as diminished phagocytic activities, compared to wild type mice. In addition our data demonstrated that G6PT-KO neutrophils exhibited an enhanced late apoptosis and necrosis.

Discussion: We have developed an inducible murine model that mimics the pathophysiology of GSD1b in terms of tissues damage, neutrophils dysfunction, and susceptibility to bacterial infection.

Conclusion: This model will be exploited to develop and test new therapeutic strategies for GSD1b.

References

1. Chou et al. Curr Opin Hematol 2010; 17: 37-42.
2. Resaz R et al. Dis Model Mech 2014; 7: 1083-91.

W4.3

IP4 IS THE SECOND MESSENGER REQUIRED FOR CA²⁺ ENTRY IN DENDRITIC CELLS THROUGH PLASMA MEMBRANE IP3R3

Laura Marongiu¹, Marco Di Gioia², Valentina Poli², Laura Sironi³, Ivan Zanon², Francesca Granucci¹

¹Department of Biotechnology and Bioscience, ³Department of Physic, University of Milano Bicocca, Milano

²Harvard Medical School, Boston, Boston - USA

Purpose: Following activation with LPS, DCs produce soluble and cell surface molecules critical for initiation and control of innate and adaptive immunity. We have previously described that following LPS exposure different NFAT members are activated in DCs. The initiation of the pathway that leads to nuclear NFAT translocation is dependent on CD14 that, through the activation of Src family kinases and PLC γ 2, leads to Ca²⁺ mobilization and calcineurin activation. Nuclear NFAT translocation is required for IL-2 production and apoptotic cell death of terminally differentiated DCs. Here we analyzed the mechanism of Ca²⁺ mobilization in DCs.

Results and Discussion: With super resolution microscopy, we demonstrated that human and mouse DCs expressed IP3R3 at the plasma membrane and that these receptors colocalized with CD14 in lipid raft. We found that the increase in cytosolic calcium concentration was due to a direct calcium influx from the extracellular space, with a mechanism dependent on IP₃R3, and required IP4 as second messenger. Indeed, abrogation of IP3R3 or IP3Kb in DCs also abrogated calcium mobilization and NFAT activation. Moreover, we demonstrated that PKC θ , recruited by DAG at the plasma membrane, is required for IP3Kb activation.

Conclusions: Our results indicate that the mechanism of calcium influx triggered by CD14 requires the activation of PKC θ and IP3Kb and therefore the production of IP4 as second messenger. The release of IP4 close to the plasma membrane leads to the opening of IP₃R3 with the consequent induction of a monophasic Ca²⁺ influx necessary to activate the downstream events that trigger NFAT dependent transcriptional program.

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W4.4

GROUP V SECRETED PHOSPHOLIPASE A2 MEDIATES THE PRODUCTION OF ANGIOGENIC AND ANTI-ANGIOGENIC FACTORS FROM HUMAN NEUTROPHILS

Stefania Loffredo¹, Anne Lise Ferrara¹, Francesco Borriello¹, Raffaella Iannone¹, Maria Rosaria Galdiero¹, Gilda Varricchi¹, Gerard Lambeau², Vincenzo Gigantino³, Pasquale Esposito⁴, Marco Cassatella⁵, Francescopaolo Granata¹, Gianni Marone¹

¹Division of Clinical Immunology and Allergy and Center for Basic and Clinical Immunology Research (CISI), ⁴U.O.C. Immunohematology and Transfusion Medicine, University of Napoli Federico II, Napoli

²Université Côte d'Azur, CNRS, Institut de Pharmacologie Moléculaire et Cellulaire, Valbonne Sophia Antipolis - France

³CNR Institute of Experimental Endocrinology and Oncology "G. Salvatore", Napoli

⁵Department of Medicine, Division of General Pathology, University of Verona, Verona

Angiogenesis, the formation of new blood vessels, plays a prominent role in inflammation and tumors. This process is sustained by the coordinated production of several angiogenic factors including Vascular Endothelial Growth Factors (VEGFs) and Angiopoietins (Angs). Secreted phospholipases A₂ (sPLA₂) are mediators involved in inflammatory diseases and tumors. Human neutrophils (PMNs) are both a source and a target of sPLA₂s. These cells release group V sPLA₂ (hGV) and can be activated by sPLA₂s to release CXCL8. We have investigated the role of hGV in the production of angiogenic factors from PMNs. VEGF-A, -B, -C, -D and Angs (Ang1 and Ang2) expression was evaluated by RT-PCR in purified PMNs. Release of VEGFs, Angs, CXCL8 was evaluated by ELISA. sPLA₂ activity was measured by EIA. PMNs constitutively express mRNAs for the proangiogenic molecules VEGF-A₁₆₅, VEGF-B₁₆₇, VEGF-B₁₈₆, and Ang1. mRNA for VEGF-A₁₂₁, VEGF-A₁₈₉, VEGF-C, VEGF-D, and Ang2 was not detected. PMNs also expressed mRNA for the anti-angiogenic factor VEGF-A_{165b}. In vitro stimulation of PMNs with hGV induced the release of VEGF-A, Ang1 and CXCL8. hGV also induced the release of VEGF-A_{165b}. Preincubation of hGV with Me-Indoxam, which blocks M-type receptor-mediated effects and enzymatic activity of sPLA₂s, abolished the release of VEGF-A, Ang1 and CXCL8 but not that of VEGF-A_{165b}. The release of VEGF-A_{165b} was reduced by preincubation of neutrophils with P11 and/or TCS 2314 two antagonist of integrin receptors (α3 and α4β1). These results indicate that hGV induced the production of both angiogenic and anti-angiogenic factors from PMNs by different mechanisms. Activation of PMNs by fMLF induced the release of hGV as well as of VEGF-A and CXCL8. Preincubation of PMNs with Me-Indoxam before stimulation with fMLF inhibited the release of VEGF-A and CXCL8. These results are compatible with the hypothesis that endogenous hGV may be involved in fMLF induced release of VEGF-A and CXCL8.

W4.5

KIR2DL3 AND THE KIR LIGAND GROUPS HLA-A-BW4 AND HLA-C2 PREDICT THE OUTCOME OF HEPATITIS B VIRUS INFECTION

Danilo Di Bona¹, Anna Ajello², Claudia Colomba³, Massimo Bilancia⁴, Giulia Accardi², Maria Filomena Caiaffa⁵, Giuseppina Candore², Luigi Macchia¹, Calogero Caruso²

¹Dipartimento dell'Emergenza e dei Trapianti d'Organo, Università di Bari Aldo Moro, Bari

²Dipartimento di Biopatologia e Biotechnologie Mediche, Università di Palermo, Palermo

³Dipartimento di Scienze per la Promozione della Salute e Materno-Infantile "G. D'Alessandro", Palermo

⁴Dipartimento Jonico in Sistemi Giuridici ed Economici del Mediterraneo: Società, Ambiente, Culture, Università di Bari Aldo Moro, Bari

⁵Dipartimento di Scienze Mediche e Chirurgiche, Università di Foggia, Foggia

Purpose: Killer immunoglobulin-like receptors (KIRs) regulate the activation of Natural Killer cells through their interaction with human leukocyte antigens (HLA). KIR and HLA loci are highly polymorphic and certain HLA-KIR combinations were found to protect against viral infections. In this study, we analyzed whether the KIR/HLA repertoire may influence the course of hepatitis B virus (HBV) infection.

Methods: Fifty-seven subjects with chronic hepatitis B (CHB), 44 subjects with resolved HBV infection, and 60 healthy uninfected controls (HC) were genotyped for KIR and their HLA ligands.

Results: The frequency of HLA-A-Bw4 ligand group was higher in CHB (58%) than subjects with resolved infection (23%) (OR, 4.67; p < 0.001), and HC (10%) (OR, 12.38; p < 0.001). Similar results were obtained for HLA-C2 ligand group, more frequent in CHB (84%), than subjects with resolved infection (70%) (OR, 2.24; p < 0.10), and HC (60%) (OR, 3.56; p < 0.01). Conversely, the frequency of KIR2DL3 was lower in CHB (81%) than in subjects with resolved infection (98%) (OR, 0.10; p < 0.05). These results suggest a detrimental role of HLA-A-Bw4 and HLA-C2 groups, associated to the development of CHB, and a protective role of KIR2DL3. A stepwise variable selection procedure based on multiple logistic regression analysis identified these KIR/HLA allotypes as the most relevant, featuring high specificity (90.9%) and positive predictive value (87.5%) for the development of CHB.

Discussion: According to the predictive model the presence of KIR2DL3 gene is highly protective (predicts resolved infection anytime is present) if only one out of the two detrimental HLA ligand group genes (HLA-A-Bw4, HLA-C2) is present, but is unable to confer protection when both HLA-A-Bw4 and HLA-C2 are present.

Conclusion: Specific KIR/HLA combinations predict the outcome of HBV infection.

Reference

1. Di Bona et al. J Viral Hepat 2017; in press.

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WORKSHOP 5 NEUROIMMUNOLOGY

W5.1

NASAL TREATMENT OF EXPERIMENTAL AUTOIMMUNE MYASTHENIA GRAVIS WITH A RECOMBINANT FUSION PROTEIN CONTAINING THE ACETYLCHOLINE RECEPTOR TCELL-EPI TOPE 146-162

Alessandra Consonni¹, Elena Rinaldi¹, Marta Miotti¹, Martina Verolin², Sapna Sharma³, Karin Schon³, Christina Lebrero-Fernandez³, Nils Lycke³, Renato Mantegazza¹, Fulvio Baggi¹

¹Neuroimmunology and Neuromuscular Diseases, Neurological Institute "Carlo Besta", Milano

²Toleranzia AB, Gothenburg - Sweden

³Department of Microbiology & Immunology, University of Gothenburg, Gothenburg - Sweden

Purpose: To evaluate the clinical efficacy of an antigen-specific recombinant fusion protein, nasal administered, in the mouse Experimental Autoimmune Myasthenia Gravis (EAMG) model.

Methods: Tolerogenic fusion proteins are formed by a mutated cholera toxin A1 subunit (CTA1R9K), a dimer of the Ig binding D region of Staphylococcus aureus protein A (DD), and the immunodominant epitope 146-162 of Torpedo AChR alpha subunit (CTA1R9K-AChR-DD). EAMG was induced in C57Bl/6 mice by immunization with 20 µg of purified TACHr in CFA and two boosts at day 30 and 60. EAMG mice were intranasally treated with 5 µg of fusion protein, according to therapeutic protocols.

Results: CTA1R9K-AChR-DD treatment was associated with a reduction of EAMG manifestations (clinical score 0.27 ± 0.1 vs 1.9 ± 0.2 in vehicle-EAMG, $n = 12$; $p < 0.001$). Reduction of anti-mouse AChR antibody levels (1.03 ± 0.25 pmol/ml vs. 2.63 ± 0.34 , $p < 0.001$) and of muscle AChR loss (0.18 ± 0.03 pmol/g vs vehicle 0.09 ± 0.02 , $p < 0.05$) were observed. Nasal treatment was associated with down-regulation of IFN γ and IL17 pro-inflammatory mRNA, and with upregulation of TGF β , IL10, FoxP3 transcripts in lymph nodes and spleens. IFN γ and IL17 reduction and IL10 increase were observed in culture supernatants from lymph node cells, stimulated with TACHr or T146-162 peptide, from nasal-treated EAMG mice.

Discussion: Innovative immunomodulatory therapies for autoimmune diseases are needed due to the poor clinical response in some patients and the severe side effects of conventional treatments. This preclinical study provides strong evidence on the efficacy of the CTA1R9K-AChR-DD fusion protein in mice EAMG. Further studies are needed to characterize the molecular mechanisms associated to the induction of AChR-specific T cells tolerance.

Conclusions: CTA1R9K-AChR-DD recombinant protein has been shown to be an effective treatment in the EAMG model, suggesting its use in clinical trial.

Reference

1. Lycke N et al. Mucosal Immunol 2010; 3: 556-66.

W5.3

FOXP3 ISOFORMS AND T REGULATORY CELL EXHAUSTION IN MULTIPLE SCLEROSIS

Manolo Sambucci¹, Francesca Gargano¹, Marco De Bardi¹, Serena Ruggieri², Veronica De Rosa³, Claudio Gasperini⁴, Giuseppe Matarese⁵, Luca Battistini¹, Giovanna Borsellino¹

¹Neuroimmunology Unit, European Centre for Brain Research, Santa Lucia Foundation, Roma

²Department of Neurology and Psychiatry, Sapienza University of Roma and Department of Neurosciences, S. Camillo Forlanini Hospital, Roma

³Neuroimmunology Unit, European Centre for Brain Research, Santa Lucia Foundation and Institute of Experimental Oncology and Endocrinology, National Research Council (IEOS-CNR), Napoli

⁴Department of Neurosciences, San Camillo-Forlanini Hospital, Roma

⁵Institute of Experimental Oncology and Endocrinology, National Research Council (IEOS-CNR) and Department of Molecular Medicine and Medical Biotechnologies, University of Napoli "Federico II", Napoli

Purpose: Previous studies have shown that expression of CD39 defines the subset of Treg cells endowed with the most powerful suppressive abilities (1, 2). To correlate the expression of FoxP3 isoforms with immune function, we determined the positivity for FoxP3 with the available anti-FoxP3 clones in combination with surface staining of CD39. As proposed by Miyara and colleagues (3), we stained cells with CD4, CD25, and CD45RA and defined three main subsets of cells within CD4+ lymphocytes: CD25highCD45RA- memory Treg, CD25+CD45RA+ naïve Treg, and CD25lowCD45RA- activated T cells. Expression of CD39, PD-1 and of FoxP3 was then evaluated in each subset.

Methods: PBMCs were collected from fresh blood of HD and MS individuals and the stained with a combination of antibodies for Flow-Cytometry staining. Samples were acquired on Cytotflex Flow-Cytometer and analyzed with FlowJo 10.

Results: Within CD4+CD25high cells, the fraction of FoxP3+CD39+ identified with FoxP3 clone 150D was significantly lower in MS patients compared to healthy donors, and express more PD-1; staining with the other antibody clones revealed lower frequencies of CD39+FoxP3+ cells in MS patients, although statistical significance was not reached. The subset which was most enriched in FoxP3+ cells, as expected, was the CD25highCD45RA- memory Treg, in both cohorts of individuals. However, MS patients showed a statistically significant reduction of these cells. Also, FoxP3 expression levels were higher in healthy individuals compared to MS patients. The analysis of CD39 expression confirmed that this marker is prevalently present on memory T reg cells, and more so in healthy individuals

Conclusions: Overall, these results indicate that the antibody clone 150D, used in combination with surface markers that define distinct Treg subsets, reveals a reduction of T cells with immunosuppressive abilities in MS patients.

References

1. Borsellino et al. Blood 2007.
2. Gu et al. Cell Mol Immunol 2016.
3. Miyara et al. Immunity 2009.

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W5.4

EARLY, NOREPINEPHRINE-DEPENDENT, ACTIVATION OF THE HEMATOPOIETIC NICHE UPON INDUCTION OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

Tiziana Vigo¹, Nicole Kerlero de Rosbo¹, Antonio Uccelli¹

¹Department of Ophthalmology, Neurology, Genetics, Maternal and child health, University of Genoa, Genova

In the bone marrow (BM), mesenchymal stem cells (MSC) contribute to the homeostasis of the hematopoietic niche through the production of factors which promote a quiescent hematopoietic stem cell (HSC) state [1]. The sympathetic nervous system negatively controls the expression of these factors through the neurotransmitter norepinephrine (NE), whose interaction with β_3 -adrenergic receptors expressed by MSC leads to the proliferation and differentiation of HSC towards more differentiated progenitors [2]. In the model for multiple sclerosis [3], experimental autoimmune encephalomyelitis (EAE), immunization with myelin antigens results in the activation of peripheral lymphoid organs where pathogenic T cells are generated. **PURPOSE:** Our goal is to define the role of the NE-dependent activation of the hematopoietic niche in the development of EAE.

Methods: Femora and thymuses were harvested at different days post immunization (dpi) with Myelin Oligodendrocyte Glycoprotein (MOG₃₅₋₅₅) and analyzed by FACS, real-time PCR and ELISA.

Results: From 3 dpi we observed a significant increase in the number of immature HSC in the BM, together with a reduced expression of MSC-specific genes controlled by NE. Analysis of common lymphoid and myeloid progenitors showed a lymphoid bias of hematopoiesis at all time points. Parallel investigation indicated an early activation of the thymus in EAE, with a burst in maturation of inherent precursors from 3 dpi resulting in an elevated number of CD4⁺ T cells, and an increase in KLS cells, presumably derived from the BM, from 7 dpi.

Conclusions: These data suggest a role for the BM hematopoietic niche in the development of EAE, and support the hypothesis that, under pathological conditions, MSC are controlled through β -adrenergic transmission to modify the peripheral immune repertoire.

References

1. Mendez-Ferrer et al. Nature 2010; 466: 829.
2. Katayama et al. Cell 2006; 124: 407.
3. Mendel et al. Eur J Immunol 1995; 25: 1951.

W5.5

NEUROINFLAMMATION AND AUTOIMMUNITY: A COMPARISON BETWEEN CEREBROSPINAL FLUID OF PATIENTS WITH NEURO-BEHÇET'S DISEASE AND MULTIPLE SCLEROSIS

Elena Bonechi¹, Alessandra Aldinucci¹, Alessandro Barilaro², Tiziana Biagioli³, Giacomo Emmi⁴, Mario Milco D'Elios⁴, Clara Ballerini¹

¹Department of NEUROFARBA, University of Firenze, Firenze

²Division Neurology 2, ³Laboratory Department, Careggi University Hospital, Firenze

⁴Department of Experimental and Clinical Medicine, Firenze

Purpose: Neuro-Behçet's disease (NBD) is a neuroinflammatory disorder occurring in 5-30% of patients affected by systemic Behçet's disease. NBD patients may show clinical and magnetic resonance features "multiple sclerosis (MS) like" that make necessary a differential diagnosis from MS. Aim of the study is to characterize cerebrospinal fluid (CSF) cytokine profile and T cell receptor (TCR) repertoire usage in NBD patients compared to MS.

Methods: Collection of CSF, serum and peripheral blood lymphocytes (PBL) of 11 NBD and 21 relapsing remitting MS (RRMS) undergoing diagnostic lumbar puncture. NBD and MS diagnosis was made in agreement with international criteria (1; 2). Measurement of CSF and serum content of MMP9, 3 chemokines and 14 cytokines by Milliplex. Flow cytometry evaluation of MMP9 production in PBL. Study of T cell clonal expansion in CSF by CDR3 spectratyping of TCR Vbeta chain.

Results: First, NBD and RRMS patients significantly differ for MMP9 content in CSF and serum. Determining the ratio between CSF and serum MMP9 concentration and normalizing it versus CSF/serum albumin ratio, we defined the "MMP9 Index"; this parameter is significantly lower in NBD than RRMS samples and permit to identify a threshold value with a specificity of 95.4% (ROC curve). Second, NBD and MS patients show a different CSF chemoattractant signature, characterized by IL8 in NBD and CXCL13 in RRMS. Third, percentage of CSF TCR clonal expansions (restrictions) in NBD and MS are comparable (30%).

Discussion and Conclusion: With this study we found that MMP9 serum concentration and MMP9 Index could be proposed as possible biomarkers, if confirmed in an independent sample, helpful to exclude the diagnosis of MS or to confirm the suspicion of NBD in the cases of NBD positive for oligoclonal bands. Differences in CSF chemoattractants suggest a diverse mechanism of CNS invasion by immune cells.

References

1. Kalra et al. J Neurol 2014.
2. Polman et al. Ann Neurol 2011.

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WORKSHOP 6 IMMUNODEFICIENCIES

W6.1 CD8+CD28-CD127^{lo}CD39+ TREG EXPANSION: A NEW PATHOGENIC MECHANISM FOR HIV INFECTION?

Daniela Fenoglio¹, Chiara Dentone², Alessio Signori³, Alessia Parodi⁴, Francesca Kalli⁴, Giorgia Nasi⁴, Monica Curto⁴, Simone Negrini¹, Edoardo Fiorillo⁵, Valeria Orrù⁵, Antonio Di Biagio⁶, Giovanni Cenderello⁷, Valentina Bartolacci⁸, Pasqualina De Leo⁹, Eugenio Mantia¹⁰, Giancarlo Orofino¹¹, Bianca Bruzzone¹², Laura Ambra Nicolini¹³, Lucia Tamarasso³, Federico Ivaldi⁴, Mauro Giacomini¹⁴, Sanjay Bhagani¹⁵, Gilberto Filaci¹

¹CEBR-DIMI, University of Genoa, Genoa

²Infectious Diseases Department, Sanremo Hospital, Imperia

³Department of Health Sciences, University of Genoa, Genoa

⁴CEBR, University of Genoa, Genoa

⁵Istituto di Ricerca Genetica e Biomedica, Consiglio Nazionale delle Ricerche (CNR), Sede Secondaria IRGB, Lanusei, Lanusei

⁶IRCCS Azienda Ospedaliero Universitaria San Martino - IST - Istituto Nazionale per la Ricerca sul Cancro, Genoa

⁷Infectious Diseases Dept, Galliera Hospital, Genoa

⁸S.M. Misericordia Hospital, Albenga, Albenga (SV)

⁹Infectious Diseases Dept, San Paolo Hospital, Savona, Savona

¹⁰Infectious Diseases Dept, SS. Antonio, Biagio, Cesare Arrigo Hospital, Alessandria, Alessandria

¹¹Infectious Diseases Dept, Amedeo di Savoia Hospital, Torino

¹²Hygiene Unit, Infectious Disease Unit, IRCCS Azienda Ospedaliero Universitaria San Martino - IST - Istituto Nazionale per la Ricerca sul Cancro, Genoa

¹³Department of Health Sciences, University of Genoa, Genoa

¹⁴CEBR-Department of Informatics, Bioengineering, Robotic and System Engineering, University of Genoa, Genoa

¹⁵Department of Infectious Diseases/HIV Medicine, Royal Free Hospital, NHS, London - United Kingdom

References

1. Chevalier MF et al. Blood 2013; 121: 29-37.
2. Whiteside TL. Cancer Microenviron 2015; 8: 201-7.
3. Filaci G et al. Autoimmunity 2011; 44: 51-7).
4. Parodi et al. Cancer Immunol Immunother 2013; 62: 851-62.

HIV-associated immunodeficiency is related to loss of CD4⁺ T cells. This mechanism does not explain certain manifestations of HIV disease such as immunodeficiency events in patients with > 500 CD4⁺ T cells/ml or occurrence of non-AIDS tumors. Hence, it is possible that other pathogenic mechanisms causing immunodeficiency may be at play during HIV infection. Little is known about the role of regulatory T CD4⁺ cells (Treg) in HIV immunodeficiency pathogenesis and studies on CD4⁺ Treg in HIV infected patients led to controversial results [1]. Interestingly, similarities in composition and function of Treg subsets between tumors and HIV infection have been highlighted [2]. The regulatory T cell compartment includes cells belonging to the CD8⁺ T cell lineage [3]. Among the various CD8⁺ Treg subsets, a subgroup characterized by the CD8⁺CD28⁻CD127^{lo}CD39⁺ phenotype has been found to be highly concentrated within the tumor microenvironment (4). By polychromatic flow cytometry we show that HIV-infected patients have elevated circulating levels of functional CD8⁺CD28⁻CD127^{lo}CD39⁺ T regulatory cells. These cells have antigen specificity against HIV proteins, suggesting their origin from HIV-specific T lymphocytes. Their frequency post anti-retroviral therapy (ART) correlates with HIV viremia, CD4⁺ T cell count and immune activation markers, suggesting their pathogenic involvement in AIDS- or non-AIDS related complications. Their increase after initiation of ART heralds a lack of virological or clinical response: hence their monitoring is clinically relevant.

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W6.2

HIV-INFECTED PATIENTS WITH LOW CD4/CD8 RATIO SHOW ALTERED EXPRESSION OF THE INFLAMMASOME COMPONENTS AIM2, PYCARD AND IL-1 β

Simone Pecorini¹, Sara De Biasi¹, Elena Bianchini², Margherita Digaetano¹, Marcello Pinti², Lara Gibellini¹, Domenico Lo Tartaro², Cristina Mussini¹, Andrea Cossarizza³, Milena Nasi¹

¹Department of Surgery, Medicine, ²Department of Life Sciences, ³Department of Medical and Surgical Sciences for Children and Adults, University of Modena and Reggio Emilia, Reggio Emilia

Purpose: Inflammasomes are macromolecular platforms able to control maturation and secretion of the proinflammatory interleukins IL-1 β and IL-18, and play a crucial role in innate immunity [2, 3]. To evaluate the role of inflammasomes in immune reconstitution, we studied mRNA expression of the main components of inflammasomes in monocytes from successful antiretroviral treated HIV+ patients with undetectable viremia but different CD4/CD8 ratio [1].

Methods: We enrolled 26 patients, 11 with a low CD4/CD8 ratio (< 0.4) and 15 with a high ratio (> 1.2). Highly purified monocytes were stimulated with LPS for 1 and 4 hours. Total RNA was extracted and reverse transcribed for the analysis of AIM2, NLRP3, NAIP, PYCARD, IL-18, IL-1 β besides 3 reference genes (ACTB, TBP and RPS18). IL-1 β and IL-18 were quantified by ELISA on supernatants from 5 patients for each group.

Results: AIM2 levels increased in both groups after 4h of stimulation, more strongly in patients with high CD4/CD8 ratio. In this group, IL-1 β mRNA levels also increased more than in patients with low ratio. NLRP3 levels increased similarly in both groups, after 1h of stimulation. PYCARD levels decreased in 4h-stimulated cells of patients with >1.2 ratio. Supernatant levels of IL-1 β increased in both groups after 4h LPS stimulation, while IL-18 remains stable.

Discussion: HIV-Infected patients with low CD4/CD8 ratio showed altered expression of the monocyte inflammasome components AIM2, PYCARD and IL-1 β . This could indicate a diminished capability to recognize nucleic acids. The presence of similar levels of cytokines may be due to the low number of patients analyzed.

Conclusions: An optimal activation of inflammasomes in monocytes could play a crucial role in the immune reconstitution. This may provide insights into the development of potential therapeutic targets.

References

1. Mussini et al, Lancet 2015.
2. Nasi et al. AIDS 2015.
3. Thompson et al. Viruses 2011.

W6.3

PRECURSOR B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA PRESENTING WITH HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS IN TWO CHILDREN WITH HETEROZYGOUS A91V MUTATION OF THE PERFORIN (PRF1) GENE

Roberta Koronica¹, Paola Muggeo¹, Giampaolo Arcamone¹, Francesco De Leonadis¹, Elena Sieni², Baldassarre Martire¹, Nicola Santoro¹

¹U.O.C. di Oncologia e Ematologia Oncologica Pediatrica, Azienda Ospedaliera Policlinico-Giovanni XXIII, Università di Bari, Bari

²D.A.I. Oncoematologia, AOU Meyer, Firenze, Firenze

Purpose: Haemophagocytic lymphohistiocytosis (HLH) is a life-threatening disorder characterized by excessive activation of T cells and macrophages and cytokine storm with overwhelming inflammation. Traditionally HLH is classified into primary (genetic/familial) or secondary form, the latter being often associated with haematological malignancies. Genetic mutations in the perforin gene give rise to approximately 30% cases of familial HLH (FLH). A frequent polymorphism A91V may impair processing of perforin protein to the active form, and has been suggested to increase susceptibility to childhood acute lymphoblastic leukemia (ALL).

Methods and Results: We recently studied two consanguineous children, male and female, who were referred at the age of 12 and 15 years respectively, because of persistent fever, cytopenia, hyperferritinemia, hypertriglyceridemia and massive splenomegaly. The patients were diagnosed with common B cell lymphoblastic leukemia on the basis of morphological and immunophenotypic study performed on the bone marrow aspirate and started to AIEOP protocol of chemotherapy for CALL. Both patients showed decreased NK cell function along with decreased perforin expressing CD8 cells and were found to be heterozygous for PRF1A91V mutation, resulting in a C to T change at position 272 in exon 2 of perforin gene.

Discussion: A91V is the most common amino-acid substitution identified in perforin, (alanine with valine at position 91), with an allele frequency ranging between 8-9% in the Caucasian population. The role of this substitution in disease pathogenesis remains unclear. Several reports have recently linked the A91V polymorphism of perforin to a variety of pathological conditions including lymphoma, acute childhood lymphocytic leukemia, as well as late-onset FHL.

Conclusions: Genetic monitoring of p.A91V mutation in pediatric patients with acute lymphoblastic leukemia presenting with hemophagocytic lymphohistiocytosis is warranted.

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W6.4

IMMUNODEFICIENCY BEHIND ENCEPHALOPATHY

Alessandra Tozzo¹, Corsini Emilio², Anna Ardisson³, Silvana Franceschetti⁴, Tiziana Granata³

¹Department of Neuropsychiatria Infantile, ²Laboratorio,

³Neuropsychiatria infantile, ⁴Neurologia, Istituto Neurologico Besta, Milano

Purpose: Autoimmune lymphoproliferative syndrome (ALPS) is a disorder of abnormal lymphocyte homeostasis with elevated level of CD4⁺ and CD8⁺ negative T lymphocytes (termed double-negative T cell- DNT cells). Clinical manifestations include noninfectious and nonmalignant splenomegaly and autoimmune pathology [1]. We report the case of a 13 years old girl, who, in addition to the expected symptoms of immune dysregulation, also manifested with bilateral and hemispheric shifting status epilepticus. Brain MRI revealed cerebral and cerebellar atrophy, while CSF analysis was unremarkable for usual autoimmune markers. Brain biopsy showed T lymphocytes infiltration.

Methods: We researched the presence of DNT cells through flow cytometry and immunophenotypic analysis and we use NGS to study ALPS related genes.

Results: Despite the negative molecular screening for ALPS, the patient fulfilled criteria for ALPS type 3 because of the chronic splenomegaly, the double negative T cells >1,5%, the autoimmune cytopenia [2]. Consistent with her immunological epilepsy the patient responded to bolus of EV steroids.

Discussion: This case suggests that peripheral trigger of neurological disease, such as the epileptic status observed in our patients, can be treated with traditional anti-inflammatory drugs. When the immune system and the brain are affected in the same patient, a common etiology should be considered.

Conclusions: Systemic immune dysregulation must be considered in the differential diagnosis of epilepsies.

References

1. Seidel MG. Autoimmune and other cytopenias in primary immunodeficiencies: pathomechanisms, novel differential diagnoses and treatment. Blood 2014; X Volume 124, Number 15.
2. Lindsey AG et al. Optimal management of autoimmune lymphoproliferative syndrome in children. Pediatr Drugs 2016; 18: 261-72.

W6.5

ANALYSIS OF THE MOLECULAR MECHANISMS OF C1-INHIBITOR DEFICIENCY INDUCED ANGIOEDEMA

Fleur Bossi¹, Roberta Bulla², Peter Spath³, Francesco Tedesco⁴, Oriano Radillo¹, Marco Cicardi⁵

¹IRCCS Burlo Garofolo, Trieste

²Department of Life Sciences, University of Trieste, Trieste

³Institut für Pharmakologie der Universität Bern, Inselspital, Bern - Switzerland

⁴IRCCS, Istituto Auxologico Italiano, Milano

⁵Department of Biomedical and Clinical Sciences Luigi Sacco, University of Milano, ASST Fatebenefratelli Sacco, Milano

Purpose: Angioedema (AE) due to inherited or acquired deficiencies of C1 inhibitor (C1-INH) is characterized by localized swelling of deeper layers of the skin or submucosal tissues, becoming particularly life threatening if it occurs in the upper respiratory tract. C1-INH regulates the release of bradykinin which can enhance permeability of post-capillary venules interacting with its receptors. The drugs currently used are more symptomatic than curative, so we sought to identify the molecular mechanisms responsible for the induction of vascular permeability.

Methods: We used a transwell in vitro model with a filter covered by primary human endothelial cells (EC), in the upper chamber we add the fluorescent-BSA and the stimuli and the BSA leaked into the lower chamber was evaluated using a Fluorescence reader.

Results: EC were incubated with plasma collected from patients during attack (APL) and the presence of C1-INH in the majority of the patients was able to block the permeability. To mimic the in vivo situation we stimulated the EC with the APL for 30 min and then the SN was collected and used to stimulate the ECs in the transwell model. In that case the inhibition of the leakage by C1-INH was not seen in all the patients. This observation was further confirmed by using the plasma collected from 1 patient before and 1 h after the clinical treatment with C1-INH, indeed there is no difference in the EC leakage induced by the plasma before and after the treatment.

Discussions: The inhibition of endothelial leakage induced by APL stimulation by C1-INH indicates the involvement of that molecule in controlling the onset of AE attacks, although the inability of C1-INH to completely block the permeabilizing effect of the SN indicates that after the activation of the cells there are other molecules involved.

Conclusion: Since the clinical treatment of AE can be done with different drugs besides C1-INH we have to analyze the most appropriate therapeutic approach.

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WORKSHOP 7 TUMOR IMMUNOLOGY I

W7.1

NEUTROPHILS EXERT AN ANTI-TUMORAL ROLE IN 3MCA-INDUCED SARCOMA CARCINOGENESIS

Andrea Ponzetta¹, Marialuisa Barbagallo¹, Martina Molgora¹, Eduardo Bonavita², Maria Rosaria Galdiero³, Elena Magrini¹, Nadia Polentarutti¹, Cecilia Garlanda⁴, Alberto Mantovani⁴, Sebastien Jaillon⁴

¹Experimental Immunopathology, Humanitas Clinical and Research Center, Rozzano

²Cancer Research UK, Manchester - United Kingdom

³Università di Napoli "Federico II", Napoli

⁴Hunimed, Humanitas University, Rozzano

Purpose: The view of neutrophil as a cell involved only in the early phases of inflammation has been challenged in the last years, so that neutrophils are now considered key players in the orchestration of the immune response. To determine neutrophil contribution to tumor development, previous studies often relied on the poorly effective antibody-based neutrophil depletion in transplantable tumor models, but rigorous in vivo evidence assessing the neutrophil role in cancerogenesis is missing.

Methods: We investigated this issue using a model of chemically-induced cancer (3-MCA induced sarcoma) and taking advantage of a genetic model of neutrophil deficiency (csf3r^{-/-} mice).

Results: Neutrophil deficiency was associated with increased susceptibility to sarcoma, and tumor microenvironment from csf3r^{-/-} mice displayed protumoral features (e.g. increased frequency of M2 macrophages, reduced IFN γ concentration and skewed T cell polarization). In addition, neutrophil density within tumor significantly correlated with reduced proliferation rate of tumor cells in immunocompetent mice. Importantly, adoptive transfer of naïve neutrophils reduced tumor growth in csf3r^{-/-} mice, restoring normal IFN γ levels. Additionally, IFN γ depletion completely abolishes sarcoma protection observed in csf3r^{+/+} mice.

Discussion: Collectively, our data indicate that genetic deficiency of neutrophils affects the anti-tumor response and is associated with increased susceptibility to chemically-induced cancerogenesis.

Conclusions: Until recently neutrophil function was mostly related to acute inflammation and defense against pathogens. We (and others) have challenged this dogma and demonstrated that neutrophils represent an essential component in the control of tumor onset and development.

W7.2

IL-18 RECEPTOR MARKS FUNCTIONAL CD8 T CELLS IN NON-SMALL CELL LUNG CANCER

Eleonora Timperi¹, Chiara Focaccetti², Daniela Gallerano², Mariangela Panetta¹, Sheila Spada¹, Paolo Visca¹, Francesco Facciolo¹, Arsela Prelaj³, Flavia Longo³, Daniele Diso⁴, Federico Venuta⁴, Paola Nisticò¹, Vincenzo Barnaba²

¹Laboratorio di Immunologia Sperimentale e Oncologia, Istituto Nazionale Tumori Regina Elena, Roma

²Dipartimento di Medicina Interna e Specialità Mediche, Roma - Italy,

³Dipartimento di Medicina Molecolare Oncologica UOC, "Sapienza" Università di Roma, Roma

⁴Dipartimento di Chirurgia Generale e Specialità Chirurgiche Paride Stefanini, Roma

Purpose: IL-18 is a pro-inflammatory cytokine, produced in response to several pathogen- or damage-associated patterns¹. It can be represented in different tumor types, but its relevance in human cancer is not clear.

Methods: The goal of our study was to characterize NSCLC-infiltrating CD8 T cells by phenotypical and functional assays from tumor site (T), normal tissue (NT) and peripheral blood (PB) of adenocarcinoma NSCLC patients and dissect the role of IL-18 in term of ability to modulate tumor-infiltrating CD8 T cell functions.

Results: Our data demonstrated an accumulation of dysfunctional CD8 T cells in T compared to NT counterpart on the basis of the following evidences: a) while both memory effector (EM) and terminally-differentiated (EMRA⁺) CD8 T cell subsets were consistently represented in NT, EMRA⁺ cells were significantly reduced in T; b) Tbet⁺Eomes⁺ cells were preferentially accumulated, and Tbet⁺Eomes⁺ cells significantly reduced in T; c) PD-1⁺ CD8 T cells were preferentially accumulated in T. Counter-intuitively, the analysis of cytokine composition in the conditioned media of NT and T, highlighted a significant increase of IL-18 and IFN- γ in T, than the counterpart. Notably, we observed that tumor cells represented the principal source of IL-18 and that CD8 T cells expressing IL-18R were especially accumulated in T. These IL-18R⁺ cells are more prone to produce IFN- γ and were more confined in Tbet⁺Eomes⁺ subpopulation, suggesting that although Tbet⁺Eomes⁺ were poorly accumulated in T, they represent a subpopulation able to respond to IL-18. Indeed, ex vivo IL-18 treatment of mononuclear cells enriched from T increased the IFN- γ production, especially by the IL-18R⁺ (Tbet⁺Eomes⁺) subpopulation.

Discussion/Conclusion: These data suggest Tbet⁺Eomes⁺, despite poorly representative in T, may represent a functional CD8 subpopulation able to produce IFN- γ by the IL-18/IL18R interaction, highlighting the importance of IL-18 for strategy to fight cancer.

Reference

1. Dinarello CA. Overview of the interleukin-1 family of ligands and receptors. Semin Immunol 2013; 25: 389-93.

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W7.3

MYCN IS AN IMMUNOSUPPRESSIVE ONCOGENE DAMPENING THE EXPRESSION OF LIGANDS FOR NATURAL KILLER CELL-ACTIVATING RECEPTORS IN HUMAN HIGH-RISK NEUROBLASTOMA

Elisa Brandetti¹, Irene Veneziani¹, Ombretta Melaiu¹, Annalisa Pezzolo², Aurora Castellano¹, Renata Boldrini¹, Elisa Ferretti², Doriana Fruci¹, Lorenzo Moretta³, Vito Pistoia³, Franco Locatelli¹, Loredana Cifaldi¹

¹Department of Pediatric Hematology and Oncology, Bambino Gesù Children's Hospital, IRCCS, Roma

²Laboratory of Oncology Giannina Gaslini Institute, Genoa

³Department of Immunology, Bambino Gesù Children's Hospital, IRCCS, Roma

Purpose: Neuroblastoma (NB) is the most common extracranial solid tumor occurring in childhood. Amplification of the MYCN oncogene is associated with poor prognosis. Down-regulation, on NB cells, of ligands recognized by Natural Killer (NK) cell-activating receptors, involved in tumor cell recognition and lysis, may contribute to tumor progression and relapse (1).

Methods: For this study, we used 12 NB cell lines and 12 primary NB samples and we performed experiments by flow cytometry analysis, western blotting, qPCR, immunohistochemical assay, NK cell degranulation and cytotoxic assays.

Results: Here we demonstrate that MYCN expression inversely correlates with that of ligands recognized by NKG2D and DNAM1 activating receptors in human NB cell lines, through a mechanism mediated by p53 and c-MYC, two transcription factors known to be involved in the regulation of activating ligand genes (2,3). In the MYCN-inducible Tet-21/N cell line, down-regulation of MYCN resulted in enhanced expression of the activating ligands MICA, ULBPs and PVR, which rendered tumor cells more susceptible to recognition and lysis mediated by NK cells. Consistent with these findings, an inverse correlation was detected between the expression of MYCN and that of ligands for NK-cell activating receptors in 12 NB patient specimens.

Discussion: Taken together, these results provide the first demonstration that MYCN acts as an immunosuppressive oncogene in NB cells that negatively regulates the expression of ligands for NKG2D and DNAM-1 NK cell-activating receptors.

Conclusion: Our study provides a clue to exploit MYCN expression levels as a biomarker to predict the efficacy of NK cell-based immunotherapy in NB patients.

References

1. Marcus A, Gowen BG, Thompson TW, Iannello A, Ardolino M, Deng W, et al. Recognition of tumors by the innate immune system and natural killer cells. *Advances in Immunology* 2014; 122: 91-128.
2. Nanbakhsh A, Pochon C, Mallavialle A, Amsellem S, Bourhis JH, Chouaib S. c-Myc regulates expression of NKG2D ligands ULBP1/2/3 in AML and modulates their susceptibility to NK-mediated lysis. *Blood* 2014; 123: 3585-95.
3. Textor S, Fiegler N, Arnold A, Porgador A, Hofmann TG, Cerwenka A. Human NK cells are alerted to induction of p53 in cancer cells by upregulation of the NKG2D ligands ULBP1 and ULBP2. *Cancer Research* 2011; 71: 5998-6009.

W7.4

LOSS OF CROSS-PRESENTATION AND INCREASED TH17-POLARIZING POTENTIAL IN SUBSETS OF LUNG RESIDENT DENDRITIC CELLS DURING CANCER GROWTH

Nicoletta Caronni¹, Francesca Simoncello¹, Karla Evelia Cervantes Luevano¹, Giulia Maria Piperno¹, Ruby Alonso Ramirez Lemoine², Olivier Lantz², Federica Benvenuti¹

¹ICGEB, Trieste

²Inserm U932, Institut Curie, Paris - France

Purpose: Despite a generally recognized loss of dendritic cell (DCs) functions in cancer, qualitative changes in DC capacities and cell-extrinsic factors causing suppression have been poorly explored. The purpose of our work is to define the alterations occurring in tissue resident DCs during cancer progression and the mechanism behind them.

Methods: Comparative analysis of tissue resident DC subsets were performed in an orthotopic Kras/Trp53-driven model of lung adenocarcinoma. Resident CD103 (cDC1) and CD11b (cDC2) were isolated from healthy or tumor-bearing lungs and their innate and adaptive functions were examined.

Results: Alterations of DC1 and DC2 frequencies and functions were observed in tumor lungs. Tumor-DC1 show modulation of transcripts related to innate activation and lysosomal proteins and are deeply inhibited in cross-presentation compared to lung-resident DC1. Tumor-DC2 efficiently present antigens to CD4+ T cells but undergo a skewing in cytokine profiles, by losing the ability to produce IL12 but enhancing IL23 production, causing a strong enhancement of their Th17 polarizing potential. We are currently screening by depletion experiments and cancer cell gene knock-out, which factors in the tumor microenvironment are responsible for these alterations.

Discussion: The comparative analysis of innate and adaptive functions of tissue resident DCs subsets in a mouse model of lung cancer demonstrate that conditioning by the tumor environment subverts subset-specific dendritic cell functions blocking cross-presentation in cDC1 and enhancing Th17 promoting functions of DC2.

Conclusion: Understanding the mechanism of DC functional suppression and the cell-extrinsic factors behind them is an essential step toward the identification of new strategies to restore a competent antigen-presenting compartment at tumor site.

References

1. DuPage M et al. *Cancer Cell* 2011; 19: 72-85.
2. Pfirschke C et al. *Immunity* 2016; 44: 343-54.

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W7.5

miRNAS PROFILE OF BONE MARROW FIBROBLASTS IN MULTIPLE MYELOMA: RELATIONSHIP WITH DISEASE PROGRESSION AND DRUG-RESISTANCE

Vanessa Desantis¹, Lucia Di Marzo¹, Ilaria Craparotta², Luca Beltrame², Sergio Marchini², Marcella Arciuli³, Fabrizio Visino¹, Francesca Calabria¹, Anna Gallone³, Maurizio D'Incalci¹, Maria Antonia Frassanito¹, Angelo Vacca¹

¹Department of Biomedical Sciences and Human Oncology (DIMO), Section of Internal Medicine and Clinical Oncology, Policlinico-Bari, Bari

²Department of Oncology, Pharmacological Research Institute Mario Negri, Milano

³Department of Basic Medical Sciences, Neurosciences and Sensory Organs, University of Bari, Bari

Purpose: microRNAs (miRs) regulate gene expression at post-transcriptional level modulating several biological processes. Bone marrow (BM) fibroblasts (or cancer associated fibroblasts, CAFs) from active multiple myeloma (MM) patients present an activated phenotype (FSP1⁺/FAP⁺/αSMA⁺), with higher proliferative rate compared to monoclonal gammopathy of undetermined significance (MGUS) CAFs¹. BM CAFs from bortezomib (bort)-resistant patients are resistant in vitro to the drug and prevent bort-induced apoptosis of co-cultured MM cells². Our purpose was to investigate whether a specific miR profile is associated to the phenotype and functional activities of BM CAFs in MGUS to MM transition and drug resistance.

Methods: miRs expression was analyzed by microarray and validated by qRT-PCR and flow cytometry on CAFs purified from BM aspirates of MGUS and MM patients. miRs target genes were identified by interrogating different tools commonly used to predict human miR gene targets and validated by western blot analysis. miRs functional effects were analyzed in CAFs transiently transfected with miRCURY LNA inhibitors and mimics.

Results and Discussion: MM and MGUS CAFs showed a different miRs profile, including 9 up-regulated and 17 down-regulated miRs. Among the over-expressed miRs, we focused on miRs showing a major significant p-value: miR-27b-3p and -214-3p. Target genes of miR-27b-3p and -214-3p were FBXW7 and PTEN, respectively, involved in cell apoptosis, proliferation and CAFs activation. Inhibition of miR-27b-3p induced the over-expression of FBXW7, an ubiquitin ligase, which negatively modulated the expression of MCL-1, NOTCH and Cyclin E1/2. miR-214-3p inhibition increased PTEN levels down-regulating the AKT/GSK3 pathway and Cyclin D1. Finally, co-cultures of MM cells with CAFs and bort treatment increased miRs expression.

Conclusions: MGUS to MM transition and drug resistance is related to a specific miRs profile. Over-expression of miR-27b-3p and -214-3p induces cell proliferation and resistance to spontaneous and bort-induced apoptosis in MM CAFs.

References

1. Frassanito MA et al. Leukemia 2014.
2. Frassanito MA et al. Leukemia 2016.

W7.6

THE INTERPLAY BETWEEN ANTI-CD20 THERAPEUTIC ANTIBODIES AND "MEMORY" NATURAL KILLER CELLS

Cristina Capuano¹, Simone Battella², Chiara Pighi², Lavinia Franchitti², Angela Santoni², Ricciarda Galandrini¹, Gabriella Palmieri¹

¹Department of Experimental Medicine, Sapienza University, Roma

²Department of Molecular Medicine-Laboratorio Pasteur Italia Fondazione Cenci Bolognetti, Sapienza University, Roma

Purpose: Our study focuses on the recently described long-lived and highly functional NK cell populations (dubbed memory NK cells), defined by the lack of expression of CD16-associated FcεRIγ chain and the ability to produce high amounts of IFNγ upon CD16 re-stimulation (1). Particularly relevant are our recent observations demonstrating that the sustained stimulation of NK cells with obinutuzumab (anti-CD20 mAb)-opsonised tumor cells leads to the selective down-regulation of FcεRIγ chain, along with the priming for enhanced IFNγ production (2). Here we want to study the capability of anti-CD20 mAbs to support memory NK cell expansion.

Methods: CD56⁺CD16⁺CD3^g (memory) and CD56⁺CD16⁺CD3^g (conventional) NK cells from healthy donors were quantified ex vivo and after 10 day co-culture with anti-CD20 mAb-opsonised CD20⁺ Raji cells in the presence of IL-2. Two different anti-CD20 mAbs, currently employed in the treatment of B cell malignancies were chosen: first generation, reference molecule, rituximab, and next generation, Fc-engineered, obinutuzumab, which shows increased binding affinity to CD16.

Results: Almost 55% of healthy donors exhibit a population of memory NK cells, accounting for 5%-70% of total peripheral blood NK cells. We observed that CD56⁺CD16⁺CD3^g (memory) NK cells selectively undergo 2- to 12-fold expansion, upon co-culturing with anti-CD20 opsonised targets, with no major differences between different anti-CD20 mAbs; on the opposite, CD56⁺CD16⁺CD3^g (conventional) NK cell proliferation is not affected by CD16 stimulation. The phenotypic and functional characterization of anti-CD20 mAb-expanded memory NK cells is under investigation.

Conclusions: Our data highlight a new aspect of the interplay between therapeutic mAbs and NK cell plasticity, suggesting a potential tool for the clinical exploitation of NK cell effector functions.

References

1. Fehniger TA et al. Trends Immunol 2016; 37: 877.
2. Capuano C et al. Oncoimmunology in press.

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WORKSHOP 8 TUMOR IMMUNOLOGY II

W8.1

GALECTIN-3 HAS AN IMMUNOSUPPRESSIVE ROLE IN THE EARLY PHASES OF PROSTATE CANCER DEVELOPMENT AND LYMPH NODE INVASION

Sara Caputo^{1,2}, Chiara Svetlana Brambillasca², Matteo Grioni²,
Elena Jachetti², Massimo Freschi³, Claudio Doglioni³,
Rossella Galli⁴, Matteo Bellone²

¹Università Vita-Salute San Raffaele, Milano

²Cellular Immunology Unit, Division of Immunology,
Transplantation and Infectious Diseases, ³Pathology Unit, IRCCS
San Raffaele Scientific Institute, Milano

⁴Neural Stem Cell Biology Unit, Division of Regenerative
Medicine, Stem Cells and Gene Therapy, Milano

Purpose: Galectin-3 (Gal-3) is a glycan-binding protein with several pro-tumoral and immunosuppressive functions. Gal-3 is already expressed in prostate intraepithelial neoplasia (PIN), and variably expressed in prostate cancer (PCa) primary lesions (1) and metastases (2). As transcriptomic analyses showed that Gal-3 was overexpressed in immunosuppressive PCa stem-like cells (CSC) colonizing prostate-draining lymph nodes of mice affected by autochthonous PIN (3), we asked if CSC and/or more differentiated PCa cells already at the stage of PIN use Gal-3 to evade immune surveillance locally and at sites of dissemination. Methods: Gal-3 expression and immunosuppressive activity was investigated in human and murine tissues, CSC and PCa cells by immunohistochemistry, immunofluorescence, FACS and ImageStream.

Results: We confirmed expression of Gal-3 in CSC, human and mouse PIN lesions and PCa cell lines. We also found expression of Gal-3 at the invading edge of lymph node metastases. Gal-3 was involved in CSC-mediated immune suppression because either Gal-3 silencing in CSC or co-culture of CSC and T cells in the presence of the Gal-3 inhibitor LacNAc rescued T cell proliferation. LacNAc also rescued the proliferation of T cells from lymph nodes of mice affected by PIN.

Discussion: We speculate that at the stage of PIN, Gal-3 participates in generating local immunosuppression, thus favoring lymph nodes dissemination.

Conclusions: We propose Gal-3 as a key molecule used by prostate CSC and PCa cells to dodge immune surveillance both locally and in lymph nodes.

References

1. Wang Y et al. Am J Pathol 2000; 174: 1515-23.
2. Nakajima K et al. Cancer Res 2016; 76: 1391-1402.
3. Jachetti E, Caputo S, et al. Cancer Res 2015; 75: 2095-108.

W8.2

ROLE OF COMPLEMENT IN CANCER-RELATED INFLAMMATION

Elena Magrini¹, Sabrina Di Marco¹, Kevin Berthenet¹,
Maria Luisa Barbagallo¹, Andrea Ponzetta¹,
Alberto Mantovani², Cecilia Garlanda²

¹IRCCS - Humanitas Clinical and Research Center, Rozzano,
Milano

²Humanitas University and IRCCS - Humanitas Clinical and
Research Center, Rozzano, Milano

Purpose: Cancer related inflammation (CRI) plays a fundamental role in fuelling tumor appearance and development [1]. Although the important contribution of complement activation to inflammation, its role in CRI and tumor progression still remains unclear. The purpose of this project is to clarify the contribution of complement activation to CRI.

Methods: In vitro C3 deposition assays and immunofluorescence staining for C3 have been performed to verify complement activation on tumor cells and tissues, respectively. C3 deposition assays were performed in presence of sera depleted of pathway-specific molecules to identify which complement activating pathway was responsible for C3 deposition on tumor cells. Experiments of mesenchymal-, induced by 3-methylcholanthrene (3-MCA), and epithelial-carcinogenesis, induced by dimethylbenz- α -anthracene/terephthalic acid (DMBA/TPA) treatments, were performed in mice deficient for the key molecule C3 and for the receptor 1 of C5a (C5aR1), an anaphylatoxin produced downstream C3 cleavage. We evaluated the tumor infiltrating immune cells by flow cytometry and the cytokine and chemokine levels by ELISA.

Results: We observed C3 deposition on human and murine cancer cell lines in vitro and on 3-MCA- and DMBA/TPA-induced tumor tissues but not on normal skin. We found that the classical pathway was the main responsible for C3 deposition on tumor cells in vitro. C3^{-/-} mice were protected from tumor growth compared to wt in both models, while C5aR1^{-/-} mice were protected from tumor development in the DMBA/TPA but not in the 3-MCA model. The protection of C5aR1^{-/-} mice in the DMBA/TPA model was associated to reduced recruitment of macrophages into tumor.

Discussion and conclusions: All together our results indicate that complement activation occurs in tumor and contributes to tumor development, although the mechanism/s implicated are different in the two models.

Reference

1. Mantovani A et al. Cancer-related inflammation. Nature 2008.

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W8.3

NATURAL KILLER (NK) CELLS AND MULTIPLE MYELOMA (MM)-DERIVED ENDOTHELIAL CELLS: MOLECULAR INTERACTIONS AND THEIR POSSIBLE SHAPING BY INTERLEUKIN-27

Beatrice Casu¹, Alessandra Dondero¹, Francesca Bellora¹, Claudia Cantoni², Silvia Gaggero¹, Angelo Vacca³, Antonella Frassanito³, Cinzia De Luisi³, Alessandro Moretta¹, Cristina Bottino², Roberta Castriconi¹

¹Department of Experimental Medicine(DIMES), University of Genoa, Genoa

²Istituto Giannina Gaslini, Genoa

³Department of Biomedical Sciences and human Oncology, University of Bari, Bari

Purpose: Angiogenesis is a hallmark of tumor progression in MM¹. We analyzed the activity of cytokine-stimulated NK cells² against endothelial cells isolated from bone marrow aspirates of MM patients (MMECs) and against normal endothelium.

Methods: Primary endothelial cells isolated by standard methods were used as targets in Cr⁵¹ release and CD107a degranulation assays. Effector cells were represented by NK cells activated by optimal or suboptimal doses of rIL-15 and rIL-27³, used alone or in combination. Flow cytometry has been used to characterize effectors cells and ECs conditioned by the mentioned cytokine mixture.

Results: MMECs were killed by NK cells activated with optimal doses of rIL-15, and HLA class I molecules had a poor protective role. Lysis was due to the cooperation of different activating receptors whose DNAM-1 played a significant role. In all endothelial cells analyzed NKG2D-ligands and B7-H6 (NKp30 ligand) were undetectable or expressed at very low levels. On the contrary MMECs expressed good levels of PVR and Nectin-2, ligands of DNAM-1; in MMECs and EA, PVR surface density was higher than in normal endothelium. rIL-27, endowed with immune-stimulatory and anti-angiogenic properties, showed the capability of up-regulating NKp46 expression and some IL-15-induced effector functions. Finally we showed that rIL-27 up-regulated PDL-2 and HLA-I on EA cell line and MMECs.

Discussion: Our results expanded the phenotypic characterization of MMECs and suggested IL-27 as adjuvant showing that it could improve the IL-15-induced activity of NK cells against MMECs. This study also highlighted the IL-27-mediated capability of up-regulating PD-L2 and HLA-I on MMECs.

Conclusions: Cytokine-activated NK cells might support conventional therapies improving the outcome of MM patients. However some crucial immune-modulatory effects of IL-27 on MMECs should not be disregarded in possible combined immunotherapeutic approaches.

References

1. Ribatti D et al. International review of cell and molecular biology 2015.
2. Vivier E et al. Science 2011.
3. Ziplat A et al. Eur J Immunol 2015.

W8.4

NK CELLS CONTROL BREAST CANCER AND RELATED CANCER STEM CELL HEMATOLOGICAL SPREAD

Rossana Talerico¹, Laura Conti², Stefania Lanzardo², Rosa Sottile³, Cinzia Garofalo¹, Arnika K. Wagner³, Maria H. Johansson³, Costanza Maria Cristiani¹, Klas Kärre³, Ennio Carbone¹, Federica Cavallo²

¹Tumor Immunology and Immunopathology Laboratory, Department of Experimental and Clinical Medicine, University "Magna Græcia" of Catanzaro, Catanzaro

²Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center, University of Torino, Torino

³Department of Microbiology, Cell and Tumorbiology (MTC), Karolinska Institutet, Stockholm - Sweden

Purpose: The growth and recurrence of a number of cancers is driven by a scarce population of cancer stem cells (CSCs), which are resistant to most current therapies¹. It has been shown previously that Natural Killer (NK) cells recognize human glioma, melanoma, colon and prostate CSCs in vitro²⁻³. We assessed whether human and mouse breast CSCs are also susceptible to NK cytotoxic activity in vitro.

Methods: Cancer stem cells (Tumorspheres) were generated by culturing in selective medium murine and human breast cancer adenocarcinoma cells. Primary tumors and lung metastases were induced by injecting BALB/c mice with either TUBO or tumorsphere-derived cells. Each group was further divided into three subgroups in which mice were either treated with tilorone, an anti-mouse-TMβ1 monoclonal antibody or PBS. After 21 days, lung metastasis formation and immune infiltrate were analyzed both by FACS and histological analysis. In vitro cytotoxicity assays effector: murine NK cells were isolated from BALB/c and C57/BL6 mice spleens, while human NK cells were obtained by peripheral blood from healthy donors. Statistical analysis was performed using either the unpaired Student's t test and ANOVA or the Mann-Whitney test. Differences in tumor incidence and survival were analyzed using Mantel-Cox log-rank tests. A p-value ≤ 0.05 was considered significant.

Results: We herein show that human and mouse breast CSCs are susceptible to NK cytotoxic activity in vitro. Moreover, CSC induced autologous NK cell activation and expansion in vivo, which correlate with the inhibition of CSC metastatic spread.

Conclusions: Our data suggest that NK cells control CSC metastatic spread in vivo and that their use in breast cancer therapy may well be fruitful.

References

1. Fornari et al. PLoS One 2014; 9:e106193.
2. Talerico et al. J Immunol 2013; 190: 2381-90.
3. Talerico et al. Front Immunol 2016; 7: 179.

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W8.5

THE PROTEIN OSTEOACTIVIN PRODUCED BY TUMOR-ASSOCIATED MACROPHAGES ACCELERATES TUMOR GROWTH AND PROMOTES CANCER CELL STEMNESS

Manuela Liguori¹, Alessandro Vacchini¹, Elena Monica Borroni¹, Fabio Pasqualini¹, Federico Simone Colombo¹, Alberto Mantovani¹, Paola Allavena¹, Cristina Belgiovine¹

¹Humanitas Clinical and Research Center, Rozzano

Purpose: Tumor-Associated Macrophages (TAM) are key orchestrators of the inflammatory tumor micro-environment, directly affecting neoplastic cell growth, neo-angiogenesis, extracellular matrix remodeling and immunosuppression. In search of novel molecules produced by tumor-conditioned macrophages, we performed a gene expression analysis and identified a highly upregulated gene: Gpnmb, coding for the protein Human Glycoprotein non-metastatic melanoma protein B (GPNMB), also named Osteoactivin.

Methods: To study the effect of this protein in tumor models in vivo, we used DBA/2J mice, which lack a functional gpnmb gene due to a spontaneous mutation, and the reconstituted strain: DBA/2J-Gpnmb⁺ mice, with the native functional protein.

Results: Osteoactivin production in macrophages is up-modulated by tumor cell supernatants, corticosteroids and IL-10, but not by pro-inflammatory stimuli, and is preferentially expressed by M2-polarized macrophages. Primary methylcholantrene-induced sarcoma generated in DBA/2J mice, and hence lacking GPNMB, had an accelerated growth and metastatic ability when transplanted DBA/2J-Gpnmb⁺ mice, where the protein was expressed by macrophages in the tumor microenvironment. Tumor cells transduced with the gpnmb cDNA grew remarkably earlier in vivo and had higher metastatic capacity compared to MOCK-transduced cells. In vitro, Osteoactivin-transduced cells survived in serum-free conditions and spontaneously formed spheroids able to self-renew. These cells expressed typical cancer stem cell markers: Sox2, CD44, c-Kit, Sca1.

Conclusions: Osteoactivin production is induced by tumor cell products in macrophages; this protein is involved in the promotion of tumor cell growth and metastasis in vivo and in the maintenance of cancer stem cell phenotype.

POSTER SESSION I

P1 - ALLERGY AND ANAPHYLAXIS

P1.1

FENNEL (FOENICULUM VULGARE) ALLERGY

Lucia Masciopinto¹, Mariangela Di Giacomo¹, Lucia Giliberti¹, Andrea Nico¹, Maria Pia Rossi¹, Marcello Albanesi¹, Danilo Di Bona¹, Maria Filomena Caiaffa¹, Luigi Macchia¹

¹Dipartimento dell'Emergenza e dei Trapianti d'Organo, Scuola e Cattedra di Allergologia e Immunologia Clinica, Università di Bari Aldo Moro, Bari

Purpose: Fennel, a typical vegetable of the Mediterranean Diet, has not been regarded as a major food allergen, so far. This project aims at estimating the occurrence of this allergy from Apulia-Southern Italy and characterizing the proteins responsible for fennel allergy.

Methods: Diagnosis of fennel allergy was made by skin prick tests (SPT), with commercial extracts and an in-house semi-purified fennel extract, and CAP RAST (Thermo Fisher) for fennel and in-house RAST-capture. To assess thermostability of allergenic proteins, prick by prick tests with raw and microwave oven-heated fennel (10', 2450 MHz) were performed. RAST inhibition experiments were performed with peach and celery extracts. SDS-PAGE Immunoblotting analysis of the semi-purified fennel extract was performed with patients sera.

Results: Allergy to fennel was diagnosed in approximately 30% of all food allergy patients (57 out of a series of 189 consecutive patients). Lip angioedema and oral itching was lamented by almost all the patients (40 out of 44). Urticaria, respiratory symptoms, including dyspnea and chest tightness and gastrointestinal symptoms were also reported. One patient experienced severe anaphylaxis. Prick-by-prick tests performed with raw and microwaved fennel provided comparable skin responses. RAST-inhibition experiments resulted negative with peach, but not with celery. Immunoblotting showed the presence of different bands (33, 45 and 50 kDa).

Discussion: Fennel allergy is highly prevalent in Mediterranean area. The thermostable allergenic proteins crossreact with other members of the Apiaceae family (celery), but not with peach, as previously suggested.

Conclusions: Fennel can be considered a major food allergen in Countries with Mediterranean Diet.

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P1.2

ALLERGY TO LIPID TRANSFER PROTEIN: GENETIC BASIS

Mariagrazia Valentini¹, Eleonora Nucera², Simona Mezzacappa², Angela Rizzi², Arianna Aruanno², Raffaella Chini², Alessandro Buonomo², Gabriele Di Sante¹, Giuseppe Migliara¹, Alessia Piermattei¹, Francesco Ria¹, Domenico Schiavino²

¹Institute of General Pathology, ²Allergy Unit, Università Cattolica del Sacro Cuore, Roma

Purpose: Allergenic LTP has been identified in pollens vegetable foods, fruits and latex. We examined the distribution of HLA-DRB1 alleles in a cohort of 96 patients suffering from food allergy, comparing 61 patients positive for LTP to 35 patients that were negative for LTP.

Methods: All patients underwent skin prick test and quantification of specific IgE with foods involved in the clinical history. Genomic DNA was extracted from peripheral whole blood samples stored at -20°C until DNA extraction and HLA typing was performed.

Results: We report that DRB1*14 was significantly decreased in LTP+ patients and DRB1*07 was significantly increased in LTP- patients, in our cohort. We found that several HLA-DRB1 alleles were specifically associated (positively or negatively) with presence of IgE specific for individual foods within the LTP+ group, and that these associations were different from those found in the LTP- group. Within the LTP+ group, both positive and negative associations between food and HLA-DRB1 alleles were co-dominantly expressed. Finally, we found that the LTPs of foods associated with the HLA-DRB1*13 allele shared a short (6-mer) peptide sequences.

Discussion: These observations were consistent with the prediction of a dominant role of HLA class II haplotype in the determination of the pattern of foods to which an LTP+ subject will develop IgE, possibly through the selection of T-cell epitopes.

Conclusions: The ability of HLA haplotype to predict or exclude the food(s) for which an allergic subject positive for LTP will produce IgE will help in the clinic management of poly-allergic individuals.

References

1. Hauser M, Roulias A, Ferreira F, Egger M. Panallergens and their impact on the allergic patient. *Allergy, Asthma & Clinical Immunology* 2010; 6: 1.
2. Boehncke WH1, Loeliger C, Kuehnl P, Kalbacher H, Böhm BO, Gall H. Identification of HLA-DR and -DQ alleles conferring susceptibility to pollen allergy and pollen associated food allergy. *Clin Exp Allergy* 1998; 28: 434-41.

P2 - AUTOIMMUNITY

P2.1

TH17 CELLS ARE ENRICHED IN SKIN-DERIVED T CELL LINES FROM HERPETIFORM DERMATITIS PATIENTS

Manuela Capone¹, Veronica Santarasci¹, Marzia Caproni², Antonino Salvatore Calabrò³, Laura Maggi¹, Alessio Mazzoni¹, Mariacaterina Rossi¹, Gianni Montaini¹, Beatrice Rossetti¹, Gabriele Lami⁴, Lorenzo Cosmi¹, Francesco Annunziato¹, Francesco Liotta¹

¹Department of Clinical and experimental medicine, ²O.U Dermatology, ³Department of Clinical Experimental and Biomedical Science "Mario Serio", University of Florence, Florence

Purpose: The aim of our study is to characterize T cells infiltrating the skin in the cutaneous manifestation of celiac disease (CD) named herpetiform dermatitis (DH). We also purpose to study antigen specific T cell response to tissue (TG2) and epidermal (TG3) transglutaminases in DH compared to CD patients.

Methods: Polyclonal T cell lines, obtained from peripheral blood (PB), skin and gut samples of 10 DH patients were evaluated by flow cytometry for membrane expression and intracellular production of Th1, Th2 and Th17 cells specific markers. Antigen specific T cell lines were induced in the presence of TG2 or TG3 from PB of 8 DH patients and compared to those of 4 CD patients.

Results: We found CD4+CCR6+ cells increased in skin and gut compared to PB. Accordingly, the frequency of IL17+ cells was higher in cultures from tissues than in those from PB. Interestingly both CD4+ and CD8+ TNFα-producing T cells resulted higher in the skin if than in PB and gut. Results on antigen specificity are very preliminary: in one DH patient we observed that TG2 expanded T cells, once re-stimulated showed high proliferation to both TG2 and TG3 while in CD patients this never occurred.

Discussion: Our data provide new evidences that Th17 cells are enriched in DH skin lesions, suggesting their involvement in DH pathogenesis. Moreover the TNFα increase at skin level could also play a crucial role. Finally results on antigen specificity led us to hypothesize that in DH patients the T cell response might be due to a TCR cross-reactivity towards TG antigens.

Conclusions: We confirm already published data about Th17 cells involvement in CD and ascribe to them a possible central role even in DH. If those preliminary data will be confirmed by our scheduled larger studies, new possible treatments should be proposed, including biological therapy, being DH very slow to respond to gluten free diet.

References

1. Sárdy M et al. PMID: 11901200.
2. Castellanos-Rubio A et al. PMID: 19127457.

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P2.2

EBV-SPECIFIC CD8+ T CELLS ARE EXHAUSTED AND SENESCENT IN MULTIPLE SCLEROSIS PATIENTS

Gisella Guerrera¹, Daniela Angelini¹, Serena Ruggieri²,
Francesca Gargano¹, Claudio Gasperini², Roberta Placido¹,
Giovanna Borsellino¹, Luca Battistini¹

¹Neuroimmunology Unit, Santa Lucia Foundation, Rome

²Department of Neuroscience "Lancisi", San Camillo Hospital,
Rome

Purpose: Multiple sclerosis (MS) is the most common chronic inflammatory disease of the central nervous system (CNS) associated with an increased Epstein-Barr virus (EBV) seroprevalence and high immune reactivity to EBV (1). While EBV infection alone cannot explain MS development, our hypothesis is that, in susceptible individuals, defects in the control of EBV facilitate the establishment of viral infection and of continuous cycles of inflammation in the CNS, due to the recruitment and activation of inflammatory cells in the brain.

Methods: To study the immune response to EBV and investigate on the effect of Glatiramer Acetate (GA) (2), we characterized the CD8+ T cells response specific for EBV lytic and latent antigens using pentamers. We measured the frequency, the activation and functional state of EBV-specific CD8+ T cells in MS patients, before and after therapy.

Results: In MS patients before therapy, a larger fraction of CD8+ cells specific for EBV latent antigens showed the phenotype of terminally differentiated and senescent cells compared to healthy individuals; the frequency of these cells did not change following treatment with GA. Interestingly, exhausted PD-1+ EBV-specific CD8 cells decreased significantly after GA therapy.

Discussion and Conclusions: At least two mechanisms are influencing the immune response to EBV in MS patients: on one hand, EBV-specific cells progress through terminally differentiated and functionally impaired and senescent cells, likely due to chronic viral stimulation, and this is an irreversible process; secondly, we find that some EBV-specific T cells are exhausted and express PD-1. This state is reversible, and the immunomodulatory activity of GA reduces their frequency and helps to restore the EBV-specific memory pool in MS patients.

References

1. Ascherio A et al. JNIP 5 2010; 271-7.
2. Weber MS et al. Neurotherapeutic 2007; 4: 647-53.

P2.3

RITUXIMAB INDUCES A REDUCTION OF LYMPHOCYTE CD3+ ACTIVATED IN PATIENTS WITH AUTOIMMUNE DISEASES

Sebastiano Cicco¹, Antonio Solimando¹, Alberto Polito¹,
Matteo Guarascio¹, Stefano Battaglia¹, Patrizia Leone¹, Franco
Dammacco¹, Vito Racanelli¹, Angelo Vacca¹

¹Dept. of Internal Medicine and Clinical Oncology, University of
Bari Medical School, Bari

B-cells play an important role in humoral immunity through differentiation into plasma cells and antibody production. Rituximab (RTX) induced B-cell depletion may change the course of systemic immune-mediated diseases (SIMD) but at this date there is no indications for the best dosage to use.

98 patients (29 M, aged 56.5 ± 14.7 years) with SIMD (19 connective tissue diseases, 52 vasculitis, 21 autoimmune cytopenias and 6 Pemphigo/pemphigoides) were off-label treated with low-dose RTX associated to Standard of Care, infused according PIRR schedule (Dammacco F Blood 2010), to obtain a disease control, steroid-sparing and reduction of other immunosuppressive drugs. Mean RTX dosage was 254 ± 130 mg/m². A complete lymphocyte count were performed before RTX treatment and at 6 months after.

Patients experienced an increase in Hb values (preRTX 11.72 ± 2.32 vs postRTX 12.76 ± 1.88 g/dl; $p < 0.02$), ESR (preRTX 38.66 ± 38.18 vs post 24.66 ± 17.94 mm1h; $p < 0.05$), and CRP (38.60 ± 17.93 vs 12.81 ± 8.09 mg/L; $p < 0.05$). No differences were found in total IgG amount (preRTX 1108 ± 37.59 vs postRTX 1140 ± 72.52 mg/dl; ns)

A significant decrease in percentage of lymphocyte CD3+HLA-DR+ (preRTX 5.795 ± 0.99 vs postRTX 3.350 ± 0.7265 ; $p < 0.05$) was found. Before RTX this percentage is directly correlated to the percentage of CD3CD8+ ($p < 0.05$) while inversely to the number of CD20 ($p < 0.05$), CD3CD4+ ($p < 0.05$) and CD45+ ($p < 0.05$). After treatment none of these correlations were found while a direct correlation to the number of CD3CD4+ ($p < 0.05$) was found. CRP reduction is directly correlated to IgG amount only in post RTX ($p = 0.001$) and ESR remains directly correlated to both preRTX ($p < 0.01$) and postRTX ($p < 0.005$).

In conclusion, low dose of RTX appears to be effective in control of SIMD at 6 months. This result could be related to a modulation in Lymphocyte CD3+ activation more than in IgG reduction. Further studies are needed to understand specific pathway involved in this mechanism.

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P2.4

FLOGOSIS AND HEART INVOLVEMENT IN LARGE VESSEL VASCULITIS

Sebastiano Cicco¹, Xhiuljano Mersini¹, Silvia Noviello¹, Anna Cirulli¹, Stefania Longo¹, Patrizia Leone¹, Stefano Battaglia¹, Franco Dammacco¹, Vito Racanelli¹, Angelo Vacca¹

¹Dept. of Internal Medicine and Clinical Oncology, University of Bari Medical School, Bari

Large vessel vasculitis (LVV) symptoms are related to increase wall thickness. Heart is usually excluded but indirect effect could determines modifications.

Aim of this study was to describe heart involvement regarding common flogosis parameters.

We analyzed 17 LVV (4 M, 54 ± 20 years). All patients underwent to laboratory analysis, ultrasound and doppler of big arteries and heart. Maximum Blood pressure (BP) measured was considered in this study.

Left ventricular hypertrophy was present in 12 patients. Among others, 3 present a concentric remodeling. 12 present a diastolic dysfunction. Aortic root normalized for body surface area was dilated (Aoi; 19.19 ± 2.52 mm/m²) and wall thickened (3.96 ± 0.83 mm). Aortic valve was regurgitant (AR) in 8.

Framingham score was $21.69 \pm 16.09\%$. Other parameters were: Uric Acid 4.04 ± 1.01 mg/dl, CRP 46.30 ± 58.33 , ESR 42.86 ± 32.38 , C3 1.19 ± 0.26 mg/dl, Systolic BP (SBP) 125.6 ± 21.28 mmHg and Diastolic (DBP) 72.5 ± 8.4 mmHg.

A direct correlation was found with SBP and Uric acid ($p = 0.04$), ESR ($p = 0.008$), diastolic dysfunction ($p = 0.02$), and Framingham score ($p = 0.03$) while was found inverse with aortic wall thickness ($p = 0.01$).

CRP was directly correlated to C3 ($p=0.02$) but inversely with aortic wall thickness ($p = 0.04$); ESR was directly correlated to C3 ($p = 0.003$), Aoi ($p = 0.03$), inversely to aortic root thickness ($p = 0.008$). Uric Acid was directly correlated to diastolic dysfunction ($p = 0.008$) and AR ($p = 0.01$). Aortic wall thickness was directly related to Aoi ($p = 0.01$) and inversely to diastolic dysfunction grade ($p = 0.009$), SBP ($p = 0.01$), uric acid ($p = 0.003$), ESR ($p = 0.008$), and CRP (0.049). Aoi was directly related to C3 ($p = 0.009$) and to wall thickness ($p = 0.01$).

In conclusion LVV could lead an increase in heart dimensions not related to systemic inflammation nor to blood pressure. On the contrary, the reduction in diastolic function should have also an inflammatory genesis. Aortic root present an increased diameter due to inflammatory status despite active flogosis did not appear the main actor in wall thickening. Uric acid appear related to vasa remodeling.

P2.5

MAIT CELLS REACT TO GUT FLORA YEASTS IN MULTIPLE SCLEROSIS

Francesca Gargano¹, Daniela Angelini¹, Barbara Serafini², Lisa Rizzetto³, Maria Chiara Buscarinu⁴, Gisella Guerrera¹, Eleonora Piras¹, Viviana Annibali⁴, Marco De Bardi¹, Serena Ruggieri⁵, Claudio Gasperini⁵, Giovanni Ristori⁴, Giovanna Borsellino¹, Duccio Cavalieri⁶, Marco Salvetti⁴, Carlotta De Filippo⁷, Luca Battistini¹

¹Neuroimmunology Unit, Santa Lucia Foundation, Roma

²Istituto Superiore di Sanità, Department of Cell Biology and Neuroscience, Roma

³Nutrition and Nutrigenomics research group Food Quality Nutrition & Health Department Research and Innovation Centre - Fondazione Edmund Mach, S. Michele all' Adige, Trento

⁴Neurology and Centre for experimental Neurological therapies (CENTERS), S. Andrea Hospital, Sapienza University, Roma

⁵Department of Neuroscience "Lancisi", S. Camillo Hospital, Roma

⁶University of Firenze, Department of Biology, Firenze

⁷CNR Institute IBIMET, Roma

Purpose: The composition of the intestinal microbiota plays a critical role in mammalian metabolism. With modern changes in diet and reduced exposure to environmental microorganisms, our immune systems which tolerates commensal microorganisms and invades pathogens appears to be out of sync with modern human lifestyles. Recent studies have suggested a role for the microbiota in immune-mediated CNS diseases such as multiple sclerosis (MS). We have previously shown that MS patients present a specific subset of proinflammatory CD8+ T cells expressing high levels of CD161 and capable of producing IL-17 [1], called mucosal-associated invariant T (MAIT) cells.

Methods: Peripheral blood mononuclear cells (PBMCs) from healthy and MS patients were isolated and analysed to detect proinflammatory cytokines. Fecal samples were collected and analyzed for microbiota diversity using the upgraded GS FLX+ platform. Activation of MAIT cells was analyzed following exposure to fungal extracts.

Results: We show a higher reactivity to yeast extracts by cells isolated from MS patients. Innate cells of the immune system produce higher amounts of proinflammatory cytokines in the MS patients. MAIT cells are more activated, proliferate and produce proinflammatory cytokines in response to yeast extracts. Also, we show that MAIT cells respond to *Saccharomyces Cerevisiae*, which is more represented in the feces obtained from MS patients.

Conclusions: CD8+ MAIT cells produce proinflammatory cytokines involved in autoimmunity. Their expansion in the peripheral blood of MS patients could be due to stimulation by gut flora strains with prominent pro-inflammatory inducing abilities, as has been shown in the mouse model [2]. Thus in MS patients an imbalance in the gut microbiota may favour the generation and amplification of proinflammatory effector cells whose effects reach way beyond the intestinal mucosa.

References

1. Annibali V et al. Brain 2011.
2. Berer K et al. Nature 2011.

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P2.6

RITUXIMAB DOWN REGULATES ANTIGEN-SPECIFIC T CELL REPERTOIRE IN MYASTHENIA GRAVIS PATIENTS

Francesco Ria¹, Mariapaola Marino¹, Alessia Piermattei¹,
Mariagrazia Valentini¹, Gabriele Di Sante¹, Amelia Evoli²,
Emanuela Bartoccioni¹

¹Institutes of General Pathology, ²Institutes of Neurology,
Università Cattolica Del Sacro Cuore, Roma

Purpose: A proportion of myasthenia gravis (MG) patients are refractory to conventional immunosuppression. B cell depletion therapy (Rituximab, RTX) has emerged as an efficacious option, a chimeric mouse/human monoclonal immunoglobulin anti-CD20 expressed on the surface of B cells. Previous findings suggested particular benefit for those patients with antibodies to muscle specific kinase (MuSK-MG) [1]. Different studies have questioned the effects also on non-B cell populations, due to the evidence that the therapeutic benefits of B cell depletion are disproportionately larger than the effects on circulating autoantibodies titers [2]. Here we tested the hypothesis of a change of T cell receptor (TCR) repertoire in MuSK-MG patients after RTX therapy.

Methods: We used the CDR3 TRBV-TRBJ spectratyping (immunoscope) to analyze the TCR specific for recombinant human MuSK protein in five HLA-DQ5+ MuSK-MG patients before and after treatment with RTX. We analyzed a specific set of four TCR VJ rearrangements (TRBV29-TRBJ2.5, TRBV28-TRBJ2.1, TRBV3-TRBJ1.2, TRBV28-TRBJ1.2) based on our previous observation of a restricted TCR repertoire [3].

Results: We confirmed that these semiprivate rearrangements were differently shared by all five patients in response to MuSK stimulation before starting Rituximab. When we analyzed the same rearrangements in samples collected after therapy, in most cases we found a Gaussian distribution without any expanded peak in response to stimulation with the antigen; this effect was more evident after six months and still evident after one year from infusion.

Conclusion: B cells are not simply antibody producers but they are also capable of antigen-presentation; in this view our results strength the hypothesis that B cell depleting therapeutic strategies could alter all aspects of B cell activity in the immune response, in particular the T and B cell crosstalk and cooperation. Alternatively, RTX could act directly on autoreactive T cell.

References

1. Diaz-Manera J. Neurology 2012.
2. Liossis SNC. Clinical Immunology 2008.
3. Marino M. J Autoimmunity 2014.

P2.7

SKIN TO BLOOD RECIRCULATION OF MEMORY CD4+ T CELLS IN PSORIASIS PATIENTS

Chiara Cozzi¹, Marco Diani², Marco Galasso³, Clara Cigni⁴,
Stefano Volinia³, Francesca Granucci⁴,
Gianfranco Altomare², Eva Reali⁵

¹Laboratory of Translational Immunology and Department
of Dermatology, ²Department of Dermatology, ³Laboratory
of Translational Immunology, I.R.C.C.S, Istituto Ortopedico
Galeazzi, Milano

³Department of Morphology, Surgery and Experimental
Medicine, University of Ferrara, Ferrara

⁴Department of Biotechnology and Biosciences; University of
Milano-Bicocca, Milano

Background and Aims: In psoriatic disease the link between the T cell subsets in lesional skin and in the circulating compartment is largely unknown [1, 2]. To investigate this aspect we dissected the phenotype of the circulating memory T cells, calculated the correlation with the clinical parameters of the disease and analyzed gene expression data in psoriatic skin lesions of independent patient cohorts.

Methods: Phenotype analysis was performed by flow cytometry on peripheral blood of 28 patients with cutaneous psoriasis and 25 healthy subjects. We analyzed the CD4⁺ and CD8⁺ T_{CM}, T_{EM} and T_{EFF} subsets for their expression of CCR6, CCR4, CXCR3, CLA, CD103 and CD69. We correlated the circulating percentage of each subset with the severity of the cutaneous manifestations measured as Psoriasis Area and Severity Index (PASI) score, and with systemic inflammation, measured as serum level of C-reactive protein (CRP). In parallel we performed a bioinformatics analysis of gene expression data in psoriatic plaques and in normal skin.

Results and Discussion: We found that the circulating fraction of CCR6⁺ CD4⁺ T_{EM} and T_{EFF} cells significantly correlated with systemic inflammation in psoriasis patients. Conversely, the percentage of CXCR3⁺ CD4⁺ T_{EM} cells negatively correlated with the PASI score, suggesting Th1 effector cell recruitment in psoriatic plaques at severe disease stages, in line with previous findings [3]. Importantly, circulating skin-tropic CLA⁺ CCR7⁺ memory CD4⁺ cells inversely correlated with the severity of the cutaneous manifestations whereas circulating CLA⁺ CD4⁺ T_{EFF} cells increased proportionally to the disease severity. Changes in the blood to skin balance of these subsets found correspondence in the results of bioinformatics analysis of gene expression in two independent cohorts of patients, showing significant increase of CCR7 expression in psoriatic plaques and its association with CLA encoding gene SELPLG, with CCR4 and with CD4 expression.

Conclusions: These findings enlighten a role for CD4⁺ T cells recirculating between skin and blood in the pathogenesis of cutaneous psoriasis and its systemic manifestations.

References

1. Lowes MA et al. Annu Rev Immunol 2014; 227-55.
2. Diani M et al. Autoimmunity Rev 2015;14: 286-92.
3. Sgambelluri F et al. J Autoimmun 2016; 70: 80-90.

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P2.8

A 5-YEAR FOLLOW UP STUDY IN SYSTEMIC LUPUS ERYTHEMATOSUS: CHANGES IN CLINICAL AND LABORATORY PARAMETERS

Chiara Cardamone¹, Roberta Parente¹, Giulia De Feo¹, Tommaso Bucci¹, Massimo Triggiani¹

¹Division of Allergy and Clinical Immunology, Department of Medicine, Salerno

Introduction: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease affecting almost every organ system. Disease phenotype at onset and during the disease is highly variable. The aim of this study was to assess the prevalence and variations of clinical manifestations and laboratory findings at the onset of the disease and during follow up.

Material and Methods: This study involved 20 patients (17 women) with SLE diagnosed according to the American College of Rheumatology (ACR) criteria. Patients were followed for a median period of five years. Disease activity was scored by SLE Disease Activity Index 2000 (SLEDAI-2K).

Results: Mean age at diagnosis was 35 ± 13 years and median disease duration was 10 ± 8 years. Sixty percent of patients were treated with immunosuppressive agents, 30% with biotechnological agents and 10% were not on treatment. During the 5 years of follow up, SLEDAI-2K score significantly decreased from 10 to 5. Arthritis and fever were more prevalent at the onset of disease vs. follow up (65% vs. 25% and 40% vs. 10%, respectively), whereas depression, xerostomia and xerophthalmia developed more frequently during the disease. Antinuclear antibodies (ANA) did not significantly change during follow up, while anti-dsDNA decrease (from 75% to 35% of patients) and became negative in 8/15 patients. Anti-ENA tend to become positive during the course, being evidenced in 14% of patients at the onset and in 29% at the follow-up. Disappearance of anti-dsDNA was associated with clinical improvement in 5/8 patients. However, in the three patients that did not improve clinically, anti-dsDNA were replaced by anti-ssA and xerostomia and/or xerophthalmia developed. Reduction of C3 (< 90 mg/dl) was found in 65% of patients at the onset and in 40% during the disease; reduction in C4 (< 10 mg/dl) was detected in 14/20 patients at the beginning of the disease and in 4/20 patients at the last follow-up. Normalization of C3 and C4 was not significantly associated with a decrease in SLEDAI score.

Conclusion: SLE is characterized by distinct evolutive phenotypes with clinical parameters changing during its course. C4 is usually consumed during the initial phase but tend to normalize rapidly. The disappearance of anti-dsDNA, rather than normalization of C4, is a reliable marker of disease activity. Substitution of anti-dsDNA by anti-ssA is associated to onset of xerostomia and/or xerophthalmia and possible evolution to secondary Sjögren's syndrome.

P2.9

IMMUNOLOGICAL TOLERANCE MECHANISMS CONTROLLING HUMAN AUTOREACTIVE CD8⁺ T CELLS IN HEALTHY AND AUTOIMMUNE CONDITIONS

Ilenia Cammarata¹, Carmela Martire¹, Alessandra Citro¹, Giovanna Peruzzi², Rossana Scrivo¹, Elisabetta Cariani³, Chiara Carone³, Guido Valesini¹, Vincenzo Barnaba^{1,4}

¹Dipartimento di Medicina Interna e Specialità Mediche,

²Istituto Italiano di Tecnologia, CLNS@Sapienza, Sapienza Università di Roma, Roma

³Ospedale Civile S. Agostino-Estense, Modena

⁴Istituto Pasteur-Fondazione Cenci Bolognetti, Roma

Purpose: We observed that autoreactive CD8⁺ T cells specific to apoptotic epitopes (AEs)¹, are accumulated as a naïve (N) phenotype in rheumatoid arthritis (RA) patients that respond to the therapy with anti-TNF- α (Rs), as well as in healthy controls (HDs), whereas as effector memory or terminally-differentiated (EM or EMRA) phenotype in patients non-responding to the therapy (NRs). We investigated the role of regulatory T cells (Tregs) that play an important role in maintaining peripheral self-tolerance.

Methods: Characterization of AE-CD8⁺ T cells and Tregs were performed by multiparametric flow-cytometry analysis. To investigate the relationship between Tregs and AE-CD8⁺ T cells we performed several functional experiments in vitro, providing the mechanistic basis of various correlations between Tregs and AE-CD8⁺ T cells in vivo. Gene expression profile of sorted N and EM-EMRA AE-CD8⁺ T cells were investigated by Nanostring technology.

Results/Discussion: Activated Tregs (actTregs) that were directly correlated with the frequency of N AE-CD8⁺ T cells, were capable to limit proliferation and differentiation of the latter. By contrast, actTregs that inversely correlated with EM-EMRA AE-CD8⁺ T cells, were unable to suppress their expansion, but rather they were killed by EM-EMRA AE-CD8⁺ T cells. This mechanism was highlighted by inverse correlation between degranulating EM-EMRA CD8⁺ T cells and actTregs. Gene expression profile of N and EM-EMRA AE-CD8⁺ T cells provided, not only different signatures, but also the molecular basis of the differential susceptibility to Treg suppression by N and EM-EMRA AE-CD8⁺ T cells

Conclusion: The naiveness of AE-CD8⁺ T cells in Rs or HDs is maintained by Tregs. Otherwise, in NRs, the cytotoxic EM-EMRA AE-CD8⁺ T cells can kill Tregs by direct or bystander mechanisms. Gene expression profile revealed the different behaviour between N and EM-EMRA AE-CD8⁺ T cells in patients, as compared with HDs.

Reference

1. Citro A. et al. PLoS One 2015.

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P2.10

CHITINASE 3-LIKE-1 IS PRODUCED BY HUMAN TH17 CELLS AND CORRELATES WITH THE LEVEL OF INFLAMMATION IN JUVENILE IDIOPATHIC ARTHRITIS PATIENTS

Manuela Capone¹, Laura Maggi¹, Veronica Santarlasci¹, Mariacaterina Rossi¹, Alessio Mazzoni¹, Gianni Montaini¹, Rolando Cimaz², Matteo Ramazzotti³, Giusi Barra⁴, Marie Pierre Piccinni¹, Raffaele De Palma⁴, Francesco Liotta¹, Enrico Maggi¹, Francesco Annunziato¹, Lorenzo Cosmi¹

¹Department of Clinical and experimental medicine, University of Firenze, Firenze

²Anna Meyer Children's Hospital and University of Firenze, Firenze

³Department of Clinical Experimental and Biomedical Science "Mario Serio", University of Firenze, Firenze

⁴Dept. of Clinical & Experimental Medicine, Second University of Napoli, Napoli

Purpose: In the present study we aim to explore chitinase 3-like-1 (CHI3L1) expression in T helper cell subsets as well as to found any possible correlation between its production and T helper cells involvement in chronic inflammatory processes.

Methods: A microarray analysis of gene expression profile was performed on Th17 and classic Th1 cell clones and CHI3L1 was found among the up-regulated genes on Th17 cells. Different types of helper T cell clones (TCCs) were then evaluated by Real Time PCR (RT-PCR) for CHI3L1 mRNA expression; protein expression was investigated in cell lysates by western blotting and in cultures supernatants by ELISA. ELISA was also used to measure CHI3L1 in the serum and in the synovial fluid (SF) of Juvenile Idiopathic Arthritis (JIA) patients.

Results: At mRNA level CHI3L1 was highly expressed by Th17, Th17/Th1, non classic Th1 and even in Th17/Th2 cell clones, whereas it was virtually absent in CD161- classic Th1 and Th2 TCCs. CHI3L1 was also detected in cell culture supernatants of Th17 and Th17-derived cells but not of classic Th1. Moreover CHI3L1 was higher in the SF than in serum of JIA patients, and it positively correlated with the frequency of Th17 and non-classic Th1 cells in SF. CHI3L1 in SF also positively correlated with the C reactive protein (CRP) serum levels, and with the levels of some proinflammatory cytokines, such as IL-6 and p40, which is the common subunit of IL12 and IL23.

Conclusions and Discussion: Here we describe for the first time CHI3L1 production by T cells owing the Th17 family. Moreover the positive correlation found between the frequency of Th17 and Th17-derived cell subsets and CHI3L1 levels in SF of JIA patients, in agreement with the suggested role of these cells in inflammatory process, candidates CHI3L1 as a possible biological target in JIA treatment.

References

1. Lee CG et al. PMID: 21054166
2. O' Shea JJ et al. PMID: 18172584

P2.11

RETROSPECTIVE AND PROSPECTIVE LONGITUDINAL EVALUATION OF A COHORT OF PATIENTS AFFECTED BY SYSTEMIC SCLEROSIS COMPLICATED BY PULMONARY ARTERIAL HYPERTENSION

Elisa Tinazzi¹, Federico Confente¹, giuseppe Patuzzo¹, Claudio Lunardi¹

¹Department of Medicine, University of Verona, Verona

Systemic sclerosis (SSc) is an autoimmune disease characterized by a complex pathogenesis which combine aspects of endothelial dysfunction, immune abnormalities, both at humoral and cellular levels, and diffuse fibrosis.

Pulmonary arterial hypertension (PAH) affects about 7-12% of SSc patients and is the major cause of death.

With the aim to analyze the prevalence of PAH in a cohort of SSc patients and to study any possible interference or relation between PAH and other clinical features or laboratoristic parameters, we enrolled 118 SSc patients for a retrospective/prospective longitudinal study. We collected SSc patients data from January 2000 to December 2016. The study included 98 females and 20 males; during the period in examination (01/2000 – 12/2016), 18 patients died, 16 females and 2 males. According to LeRoy and Medsger's criteria patients were classified as affected either of limited or diffuse SSc (respectively lSSc or dSSc). Considering the subset of the disease, 78 patients (65,7%) were affected by lSSc and 40 patients (34,3%) by dSSc. The following data of each patient involved in this study ta were collected: age, sex, cutaneous fibrosis (estimated confronting modified Rodnan Skin Score), positivity of ANA, anti-centRome antibody and anti-topoisomerase II antibody, and specific clinical manifestations such as presence of digital ulcers (active or past), involvement of digestive system, interstitial lung disease and pulmonary hypertension.

We focalized our attention on the group of patients affected by pulmonary hypertension and analysed the results of all instrumental examinations such as transthoracic rest echocardiographic test, right heart catheterization, high resolution chest CT, pulmonary function tests, 6 minute walking test at time of diagnosis and in the follow-up, particularly after the beginning of either endothelin-1 receptors antagonists (ERAs) or 5 phosphodiesterase inhibitors (5PDE-i).

In particular we considered these patients with the aim of evaluate any clinical feature or laboratoristic items which may have a predictive value for the onset of the clinical manifestation or a prognostic value of the same organ involvement.

Our data showed a significant correlation between the onset of PAH and high levels or rapid increase of ANA title and/or presence of anticentRome pattern. Furthermore, the "active" and "late" capillaroscopic pattern as well as the presence of hystory of skin ulceration or active digital ulcers are also associated with the onset of IAP. Our study also confirms that an earlier diagnosis of PAH lead to an early treatment with positive effect on quality of life and/or prognosis.

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P2.12

RAI SIGNALING IN ASTROCYTES PLAYS A CRITICAL ROLE IN SHAPING THE CENTRAL NERVOUS SYSTEM MICROENVIRONMENT DURING EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

Domiziana De Tommaso¹, Cristina Ulivieri¹, Francesca Finetti¹,
Giuliana Pellicci², Mario Milco D'Elios³,
Clara Ballerini⁴, Cosima Tatiana Baldari⁵

¹Department of Life Sciences, University of Siena, Siena

²Department of Experimental Oncology, European Institute of
Oncology, Milano

³Department of Experimental and Clinical Medicine,

⁴Department of Neurosciences, University of Firenze, Firenze

Purpose: Multiple sclerosis (MS) is an autoimmune disease where encephalitogenic Th1/Th17 cells have been long considered responsible of the neuroinflammatory reaction. Recent data indicate however that the local central nervous system (CNS) microenvironment, which is controlled by both infiltrated autoreactive T cells and CNS resident cells, plays a crucial role in disease onset and progression. Among CNS resident cells, astrocytes actively modulate the autoimmune response during MS shaping T cells responses, however, molecular mechanism underlying this function are still poorly defined¹. We have recently identified Rai as a novel astrocytic adaptor responsible for the TrkB- and IL-17R-dependent production of proinflammatory mediators in astrocytes, whose loss in mice ameliorates the experimental autoimmune encephalomyelitis (EAE), in face of higher frequency of CNS infiltrated Th17 cells highlighting the key role played by astrocytes during EAE².

Methods: To investigate the mechanism through which Rai-/- astrocytes modulate the function of encephalitogenic T cells we have used both culture supernatants from encephalitogenic T cells and co-cultures of Rai^{+/+} and Rai^{-/-} astrocytes with T cells to measure the expression of ATP hydrolyzing enzymes CD39 and CD73 on astrocytes as well as the release of cytokines from these cells.

Results and Discussion: Here we show that Rai^{-/-} astrocytes release higher level of Th17-inhibitory cytokines, IL-10 and IL-27 and upregulate CD39 and CD73 expression which are responsible for the conversion of ATP into the immunosuppressive molecule adenosine more efficiently compared with control astrocytes following exposure to culture supernatants indicating that not only can astrocytes directly affect Th17 cells but generally modulate T cell responses through contact independent mechanism.

Conclusions: Rai deficiency in astrocytes contributes to establish an unfavourable microenvironment for CNS-infiltrated T cells during EAE.

References

1. Correale and Farez Front. Neurol 2015; 180.
2. Ulivieri et al. J Immunol 2016; 480-90.

P2.13

EFFECT OF BELIMUMAB TREATMENT ON REFRACTORY SYSTEMIC LUPUS ERYTHEMATOSUS: CLINICAL AND BIO- LOGICAL RESPONSE

M. Prete, P. Leone, M.A. Frassanito, V. Desantis, S. Cicco,
V. Racanelli, A. Vacca

Department of Biomedical Sciences and Human Oncology. Unit
of Internal Medicine, University of Bari Medical School, Bari

Purpose: To evaluate the effect of belimumab on clinical, serological and immunological parameters of refractory systemic lupus erythematosus (SLE) patients and its association with disease activity.

Methods: 25 active SLE patients, according to the American College of Rheumatology classification, were treated with belimumab (10 mg/kg/day, at days 0, 14, 28 and every 28 days). Ten healthy subjects were enrolled as control group. The mean±SD follow-up was 18±6 months. We also performed flow cytometric analysis of studied T CD4+ cell subpopulations to assess Treg and Th17 subsets at T0, and after six months of treatment (T6). Disease activity was measured with the SELENA-SLEDAI index.

Results: In all SLE patients treated with belimumab a significant reduction of SELENA-SLEDAI score (13.66 vs. 5.13, p = 0.012) and of the anti-dsDNA antibody levels (87.69 vs. 11.11 p = 0.028) were observed after six months. An increase of serum C3 (0.75 vs. 1.04, p = 0.05) and C4 (0.15 vs. 0.4 p=0.028) and a significant reduction of the prednisone dosage per os (17.08 vs. 5.94, p=0.018) were also obtained. Flow cytometric analysis revealed that at T0 patients presented significantly reduced values of CD4⁺FoxP3⁺ compared to the controls (0.22±0.12% vs. 0.83±0.2%, p<0.0001) whereas at T6, a significant increase of Tregs was observed reaching values overlapping those of healthy subjects (1.04±0.22). The Th17 lymphocytes' frequency was significantly increased in patients with active SLE at T0 (3.5±2.5% vs. 1.4±0.21%, p<0.0002) and reduced at T6 (2±2.2%, p<0.001). Functional in vitro studies showed that the inhibitory capacity of Tregs purified from patients who received Belimumab was similar to that of Tregs purified from healthy subjects.

Conclusion: Belimumab treatment showed a significant reduction of the disease activity in patients with active SLE. A influence of anti-Blys therapy of regulation Treg /Th17 population homeostasis and restoration of their functional state was clearly documented. These changes may represent an pivotal target to inhibit the abnormal self-tolerance in SLE.

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P3 - B CELL ITEMS

P3.1 REGULATORY ATYPICAL B CELLS ARE EXPANDED IN PATIENTS WITH ACTIVE TUBERCULOSIS

Valentina Orlando¹, Marco Pio La Manna¹, Nadia Caccamo^{1,2},
Francesco Dieli^{1,2}

¹Central Laboratory of Advanced Diagnosis and Biomedical
Research (CLADIBIOR), A.O.U.P. Paolo Giaccone, Palermo

²Dipartimento di Biopatologia e Biotecnologie Mediche,
Università di Palermo, Palermo

Purpose: Tuberculosis (TB) is one of the most significant infectious causes of mortality and morbidity worldwide. While cellular immunity is critical in the containment of the infection, the role of humoral immunity is not yet clear. We have analyzed the frequency and the phenotype of B cells⁽¹⁾, focusing in particular on the balance between regulatory B cells (Breg) and proinflammatory B cells (Binf).

Methods: PBMC of latent TB infected subjects (LTBI), active TB patients and healthy Donors (HD) were stimulated with ionomycin and PMA for 6 hrs and subsequently surface stained with anti-CD19, -IgD and -CD27 and intracellularly with anti-IL-10 and -GM-CSF, acquired on a FACS Canto II flow cytometer and analyzed using FlowJo software. Statistical analysis was performed by Graph Pad.

Results: A lower frequency of B cells was found in active TB patients respect to HD and LTBI subjects, but not reaching statistical significance. We have observed an increase of the ratio Breg/Binf in active TB compared to HD due to a slight increase of the percentage of IL-10⁺ B cells and a decrease of GM-CSF⁺ B cells. Moreover, we have found a statistical significance differences of the ratio between active TB and HD with an altered value in memory and atypical B cells. In details, we have analysed the ratio between the subset of IgM memory and atypical B cells as IL10⁺ and GM-CSF⁺ producers, we found a significant reduction of the ratio in the former due to a remarkable decrease of percentage of IL10⁺ B cells, while an increase of the ratio in the latter mainly due to the increase of the IL10⁺ B cells.

Discussion: The altered ratio of the frequencies of Breg and Binf in the IgM memory and atypical B cell subsets of active TB patients could be involved in host immunity during M. tuberculosis infection.

Conclusions: B-cell dysfunction during TB disease could have consequences for T-cell activation, contributing to TB disease.

Reference

1. Joosten SA et al. PLoS Pathog 2016; 12: 6.

P3.2

A CONSOLIDATION PHASE IN IMMUNE MEMORY

Francesca Mantile¹, Angelo Capasso¹, Antonella Prisco¹,
Piergiuseppe De Berardinis²

¹Institute of Genetics and Biophysics, CNR, Napoli

²Institute of Protein Biochemistry, CNR, Napoli

Purpose: The mechanisms leading to the differentiation of B cells from naive to many effector cells, responsible for long-lasting protection, remain unclear. As consequence, to ensure the induction of immune memory among vaccinated, repeated injection of vaccines are required, with the better prime/boost strategies found empirically. We set out to determine how long after the administration of the first dose of vaccine the immune system becomes competent to establish a long-lasting antibody titer and to mount a memory response.

Methods: We analysed the time-course of the antibody titer to Ab epitope, and to the carrier, elicited by vaccination. We analyzed the effect of booster doses administered at early time points after the first dose both on serum antibody titer or on the immune memory.

Results: After the first injection of antigen we identified an early time-window in which the second dose can elicit different effects on the primary immune response or on the late memory response.

Discussion: Long-term immunity is achieved with the establishment of long-lasting high levels of serum antibody and with the development of memory B cells. The knowledge of mechanisms and timing of differentiation of b cells could help to better define the optimal vaccination-schedule, improving vaccine efficacy and reducing the time spent in developing new vaccines.

Conclusions: Immune memory undergoes a consolidation phase defined by a fragile equilibrium. A second dose of vaccine in this phase can disrupt the natural differentiation of memory b cells.



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P3.3 ABSTRACT WHITDRAWN

P3.4 INHIBITION OF MAMMALIAN TARGET OF RAPAMYCIN (MTOR) THROUGH THE DUAL MTOR INHIBITOR PP242 AS ANTIANGIOGENIC STRATEGY IN MULTIPLE MYELOMA Aurelia Lamanuzzi¹, Ilaria Saltarella¹, Beatrice Nico², Domenico Ribatti^{2,3}, Angelo Vacca¹, Roberto Ria¹

¹Department of Biomedical Science, Section of Internal
Medicine, University of Bari Aldo Moro Medical School, Bari

²Section of Human Anatomy and Histology, Department of
Basic Medical Sciences, Neurosciences and Sensory Organs,
University of Bari Aldo Moro Medical School, Bari

³National Cancer Institute Giovanni Paolo II, Bari

Purpose: The mammalian target of rapamycin (mTOR) is an intracellular serine/threonine kinase that forms two distinct molecular complexes. mTORC1 binds RAPTOR and triggers protein synthesis and blocks autophagy. mTORC2 interacts with RICTOR, essential to mTORC2 assembly and functionality, and promotes tumor progression, survival, migration and actin reorganization as well as it increases resistance to drugs through Akt activation. In this work we studied mTOR expression and its activation in bone marrow endothelial cells of multiple myeloma (MM) patients (MMECs) and Monoclonal Gammopathies of Undetermined Significance patients (MGEs) and mTOR involvement in MM angiogenesis also testing a new dual inhibitor of mTOR PP242 that blocks both complexes.

Methods: We studied mTOR pathway evaluating total RAPTOR and RICTOR, and total and activated mTOR and its substrates at protein and mRNA levels by western blot and real time-RT-PCR, respectively. The angiogenic effects have been studied in vitro through functional assays as wound healing, chemoinvasion and chemotaxis assays, Matrigel® assay and adhesion through Calcein AM assay. Indeed, to show the actin reorganization, we valued cytoskeleton structure with immunofluorescence exploiting the binding affinity of phalloidin to actin. Besides MMPs and angiogenic cytokines secreted by MMECs have been evaluated through in order zymography and ELISA cytokine assays. In vivo, we underlined MMECs angiogenic ability using CAM assay.

Results: MMECs present a higher activation of mTORC2 than MGEs promoting angiogenesis. This was also supported by knock-down of RICTOR where it caused the loss of MMECs angiogenic abilities in vitro. Indeed, as result of RICTOR silencing we observed mTORC1 activation, thus we opted to use PP242. The treatment with PP242 exhibited antiangiogenic activity in vitro (as RICTOR knock-down) and in vivo. Besides, PP242 synergized with bortezomib and lenalidomide reducing network of capillary-like structures on Matrigel®.

Discussion: mTORC2 is mainly involved in MMECs angiogenic abilities which are inhibited by PP242 as in vitro as in vivo. Besides, combining PP242 with MM drugs led to synergistic antimyeloma effects.

Conclusions: Our results support the idea that mTOR could be a new target in MM and PP242 may be a new drug for the therapy.

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P4 - IMMUNODEFICIENCIES

P4.1

INCREASED PLASMA CONCENTRATIONS OF ANGIOGENIC AND LYMPHANGIOGENIC FACTORS IN PATIENTS WITH HEREDITARY ANGIOEDEMA WITH C1-INH DEFICIENCY

Anne Lise Ferrara¹, Stefania Loffredo¹, Maria Bova¹, Angelica Petraroli¹, Chiara Suffritti², Francesco Boriello¹, Andrea Zanichelli², Raffaella Iannone¹, Maria Rosaria Galdiero¹, Gilda Varricchi¹, Henriette Farkas³, Massimo Triggiani⁴, Marco Cicardi², Gianni Marone¹

¹Department of Translational Medical Sciences and Center for Basic and Clinical Immunology Research (CISI), University of Napoli Federico II, Napoli

²Department of Biomedical and Clinical Sciences Luigi Sacco, University of Milano, Luigi Sacco Hospital, Milano

³Hungarian Angioedema Center, 3rd Department of Internal Medicine, Semmelweis University, Budapest - Hungary

⁴Division of Allergy and Clinical Immunology, University of Salerno, Salerno

Hereditary Angioedema with C1 Inhibitor Deficiency (C1-INH-HAE) is a rare inherited genetic disease characterized by recurrent acute swelling episodes of the skin, gastrointestinal tract and upper airways resulting from increased vascular permeability. Reduced activity of C1-INH may result in an instability of kinin pathway with the generation of bradykinin resulting in increased vascular permeability. Bradykinin increases the release of nitric oxide and Vascular Endothelial Growth Factor (VEGF) from endothelial cells. VEGF, Angiopoietin 1 (Ang1) and Ang2 are released at sites of inflammation and/or angiogenesis regulating vascular permeability. The pathogenesis of C1-INH-HAE is not completely elucidated. The aim of this study was to analyze the plasma levels of VEGFs and Angs in patients with C1-INH-HAE. 68 healthy controls, 128 C1-INH-HAE patients in remission and 15 C1-INH-HAE patients during attack were studied. Levels of angiogenic (VEGF-A, Ang1, Ang2) and lymphangiogenic (VEGF-C) factors were evaluated by ELISA. Functional assay of C1-INH was assessed by EIA kit.

Levels of VEGF-A, VEGF-C, Ang1 and Ang2 were higher in C1-INH-HAE patients in remission than in controls. The levels of VEGF-A are correlated to the decrease of functional activity of C1-INH. Interestingly, a correlation was found between the number of attacks and both VEGF-A, VEGF-C and Ang2 levels. VEGF-A, VEGF-C and Ang2 levels were not increased during attack compared to basal condition. By contrast, Ang-1 levels (an vascular stabilizer) were increased and the ratio Ang2/Ang1 (an index of vascular permeability) was decreased.

The results of this study indicate that the levels angiogenic/lymphangiogenic factors that alter vascular permeability were increased in patients with C1-INH-HAE in remission and correlate with severity disease. This condition might predispose to angioedema attacks. Moreover, the levels of these mediators, excluding Ang1, were not modified during acute attack.

P4.2

MONOCYTES AND POLYMORPHONUCLEAR LEUKOCYTES FUNCTION IN PATIENTS WITH COMMON VARIABLE IMMUNE DISORDERS ON REPLACEMENT TREATMENT WITH INTRAVENOUS IMMUNOGLOBULIN

Alessandro Prezzo¹, Filomena Monica Cavaliere¹, Caterina Bilotta¹, Metello Iacobini², Isabella Quinti¹

¹Department of molecular medicine, Sapienza Università di Roma, Roma

²Department of Pediatrics, Sapienza Università di Roma, Roma

Common Variable Immune Disorders (CVID) is a heterogenous group of primary immunodeficiency that encompass several immune dysregulations in adaptive and innate compartment of immunity. CVID is characterized by hypogammaglobulinemia, which leads to recurrent bacterial infections, autoimmunity, cancer and impaired specific antibody response to vaccines [1-3].

Purpose: To evaluate the innate immunity in CVID patients by the analysis of monocytes and polymorphonuclear neutrophils phenotype and function and to evaluate ex vivo the effects of intravenous immunoglobulin administration.

Methods: Monocytes and polymorphonuclear neutrophils (PMNs) receptors expression, phagocytosis and oxidative burst were evaluated by flow cytometry. IL-8 plasma dosage was evaluated by ELISA assays.

Results: CVID showed an expansion of the intermediate monocytes subset, an increased expression of CD11b and Siglec 9 receptors and normal phagocytosis and respiratory burst functions. Neutrophils had no alterations on phenotype and function. Similarly than in HD, IL-8 levels rapidly increased after E. coli stimulation in CVID. IVIg infusions reduced the frequency of intermediate monocytes, the expression of CD11b and Siglec 9 on monocyte and the expression of CD181 on PMN. IVIg administration did not affect the monocytes and PMN ability to upregulate their receptors after E. coli, while it slightly reduced the monocyte's phagocytosis and oxidative burst.

Discussion: The expansion of intermediate monocytes might contribute to the inflammatory status of CVID. IVIg infusion exerted an anti-inflammatory effect by reducing the intermediate subset and by diminishing the monocytes' phagocytosis and oxidative burst, even if these functions remained efficient.

Conclusions: We showed that in CVID patients the IVIg infused at replacement dosage exerted in vivo an anti-inflammatory effect on monocytes.

References

1. Bonilla FA, Barlan I, Chapel H, Costa-Carvalho BT, et al. Warnatz, International consensus document (ICON): common variable immunodeficiency disorders. J Allergy Clin Immunol Pract 2016; 4: 38-59.
2. Chapel H, Cunningham-Rundles C Update in understanding common variable immunodeficiency disorders (CVIDs) and the management of patients with these conditions, Br J Haematol 2009; 6: 709-27.
3. Quinti I, Di Pietro C, Martini H et al. Health related quality of life in common variable immunodeficiency. Yonsei Med J 2012; 53: 603-10.

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P4.3

THE LACK OF BTK DOES NOT IMPAIR MONOCYTES AND POLYMORPHONUCLEAR CELLS FUNCTION IN X-LINKED AGAMMAGLOBULINEMIA

Filomena Monica Cavaliere¹, Alessandro Prezzo¹, Caterina Bilotta¹, Metello Iacobini², Isabella Quinti¹

¹Department of molecular medicine, ²Department of pediatrics, Sapienza Università di Roma, Roma

X-linked agammaglobulinemia (XLA) is a primary immune deficiency caused by mutations in the Bruton's tyrosine kinase (BTK) gene located on X-chromosome coding for the cytoplasmic BTK expressed in adaptive and innate immune cells [1-3].

Purpose: To evaluate if in XLA patients the lack of BTK might alter the phenotype and functions of monocytes and polymorphonuclear neutrophils (PMN).

Methods: Peripheral blood monocytes and PMN frequency, receptors expression, migration, phagocytosis and oxidative burst functions and involvement of Ca²⁺ mobilization were evaluated by flow cytometry. PMN elastase and IL-8 plasma were evaluated by ELISA.

Results: XLA showed an expansion of the intermediate monocytes. Monocytes and PMN, showed a normal receptor expression with preserved migration, phagocytosis and respiratory burst functions. Ca²⁺ chelation did not affect the phagocytosis while it strongly reduced monocytes and PMN oxidative burst. Moreover, we observed an efficient Ca²⁺-independent activation of PKC. Similarly than in HD, IL-8 levels and elastase release rapidly increased after E. coli.

Discussion: Despite the lack of BTK, monocyte and PMN maintained a functional killing when FCγR are engaged. Thus, BTK was dispensable for the oxidative burst, despite it was shown to be have a role in Ca²⁺ mobilization [3]. Alternative ways for Ca²⁺ mobilization might bypass the lack of BTK. The efficient Ca²⁺-independent activation of Protein kinase C (PKC) could be an additional mechanism partially substituting the absence of BTK.

Conclusions: The lack of BTK did not alter the monocyte and PMN functions. This finding has implications for excluding additional infectious risks in patients with XLA and in patients with lymphoproliferative and autoimmune diseases treated with BTK inhibitors [3].

References

1. Suri D, Rawat A, Singh S. X-linked Agammaglobulinemia. Indian J Pediatr 2016; 83: 331-7.
2. Chear CT, Ripen AM, Mohamed SA, Dhaliwal JS. A novel BTK gene mutation creates a de-novo splice site in an X-linked agammaglobulinemia patient. Gene 2015; 560: 245-8.
3. Ren L, Campbell A, Fang H, et al. Analysis of the Effects of the Bruton's tyrosine kinase (Btk) Inhibitor Ibrutinib on Monocyte Fcγ Receptor (FcγR) Function. J Biol Chem 2016; 291: 3043-52.

P4.4

ANTIBIOTIC PROPHYLAXIS IN PRIMARY ANTIBODY DEFICIENCY PATIENTS: STUDY DESIGN

Cinzia Milito¹, Federica Pulvirenti¹, Stefano Tabolli², Maria Carrabba³, Giovanna Fabio³, Maria Cristina Pietrogrande³, Francesco Cinetto⁴, Carlo Agostini⁴, Alessandro Plebani⁵, Andrea Matucci⁶, Antonio Pecoraro⁷, Giuseppe Spadaro⁷, Baldassarre Martire⁸, Isabella Quinti¹

¹Department of Molecular Medicine, Sapienza University of Roma, Roma

²IDI, Roma

³University of Milano, Milano

⁴University of Padova, Padova

⁵University of Brescia, Brescia

⁶University of Firenze, Firenze

⁷University of Napoli, Napoli

⁸University of Bari, Bari

Purpose: Primary antibody deficiencies (PAD) are characterized by increased susceptibility to infections. Early diagnosis and treatment reduce morbidity and mortality. Immunoglobulins replacement therapy reduces the risk of acute respiratory infections, but has low efficacy to reduce lung complications. PAD patients with respiratory infections who develop lung damage may take advantage of a more aggressive treatment: antibiotic prophylaxis of infectious episodes and respiratory rehabilitation. At now, data on antibiotic prophylaxis are uncertain.

Methods: We conducted a multi-center randomized placebo-controlled-double-blind trial on 89 patients with PAD (X-linked agammaglobulinemia or Common variable Immunodeficiency) and COPD with recurrent exacerbations. All patients had spirometrically confirmed COPD. Written informed consent was obtained. The aim of the study was to evaluate efficacy and safety of azithromycin low-dose (250 mg 3 times a week for 3 consecutive days) for 24 months vs placebo. In azithromycin group we expect a decrease of COPD exacerbations (reduction of dyspnea, cough, sputum volume, purulence), no use of additional antibiotics, an increase of respiratory volumes (FEV1), an improvement of the Health Related Quality of Life measures.

Results: The study started on June 2014 and has been lasted 30 months (24 months of therapy, 6 months of follow-up). Monthly we evaluate: lung function by FEV1 and St. George's Respiratory Questionnaire, sputum sample for microbiological assessment, routine and immunological blood test, diary card for use of additional antibiotics, SF-36 Questionnaire for quality of life, the report of adverse events. Our study ended on December 2016; 83 patients have concluded the study. During the study we observed 14 drop out (9 patients withdrew informed consent; 5 patients died: 2 for respiratory distress; 1 for gastric cancer, 1 for Parkinson disease, 1 for stroke).

Discussions: To our knowledge our study is the first one on antibiotic prophylaxis in PAD patients. Conclusions: We will present our results during the meeting.

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P4.7

IgM AND IgA ANTI-PNEUMOCOCCAL CAPSULAR POLYSACCHARIDES AS PROGNOSTIC TOOL FOR COMMON VARIABLE IMMUNODEFICIENCY: A LONGITUDINAL STUDY

Federica Pulvirenti¹, Filomena Monica Cavaliere¹, Cinzia Milito¹, Rossella Carello¹, Anna Maria Pesce¹, Isabella Quinti¹

¹Department of Molecular Medicine, Sapienza University of Roma, Roma

Introduction: The clinical spectrum of CVID ranges from a poorly symptomatic form to severe phenotypes characterized by high susceptibility to infections, autoimmunity, granulomatous inflammation, lymphoproliferative disorders, and malignancies. Due to high prognosis heterogeneity, prognostic factors are required.

Objectives: With the aim to identify additional prognostic factors, we evaluated the anti-polysaccharide IgA and IgM responses by Elisa assay in 75 CVID in a longitudinal study over a 6-year period. Patients were immunized at baseline with the 23-valent pneumococcal polysaccharide vaccine (Pneumovax®). Twenty healthy donors (HD) were also included.

Results: As expected, CVID patient had lower IgM/IgA response than HD. For CVID, four immunological phenotypes were identified by post-vaccination IgM and IgA levels: IgM/IgA responders (16%), IgM-only responders (21%) and non-responders (63%). During the follow up, concomitant CVID-related conditions, immunoglobulin serum levels, respiratory infections and outcome were recorded by medical files. CVID non-responders and IgM-only responders developed more frequently respiratory infections, gastro enteric symptoms, and autoimmune manifestation in comparison to IgM/IgA responders (respectively, pneumonia: 64%, 31% and 0%; chronic diarrhoea: 25%, 14% and 0%; autoimmunity 41%, 29% and 0%; autoimmune cytopenias: 17%, 8% and 0%). Malignancies were found more frequently in the non-responders and IgM-only responders groups in comparison to IgM/IgA responders (respectively, 23%, 14% and 0%). Eleven (15%) patients died during the study time. Survival analysis according to the IgM/IgA responder status showed that the 6-years estimated survival for non-responders vs IgM-only vs IgM/IgA responders was respectively after one year 98%, 87% and 100%; after two year: 93%, 87% and 100%; after three years: 91%, 80% and 100%; after 4 years: 87%, 80% and 100%; after 5 years: 87%, 80% and 100%; after 6 years: 83%, 80% and 100%. Interesting, in our series only two deaths were due to infective complications: five were consequent to malignancies, one to autoimmune cytopenias and three to not-CVID related conditions.

Conclusions: In conclusion, even if patients could not raise the protective humoral level, in CVID the anti-polysaccharide IgA and IgM responses could represent a prognostic factor, individuating groups of patients with less immunological impairment, lower risk of comorbidities and better survival.

P4.6

CLINICAL FEATURES OF IDIOPATHIC NON CIRRHOTIC PORTAL HYPERTENSION IN PRIMARY ANTIBODY DEFECTS: NATURAL HISTORY, IMMUNOLOGY AND NEW PERSPECTIVES FOR AN UNRECOGNIZED COMPLICATION

Federica Pulvirenti¹, Cinzia Milito¹, Michele Valente², Giulia d'Amati², Oliviero Riggio³, Iaria Pentassuglio³, Isabella Quinti¹

¹Department of Molecular Medicine, ²Dpt of Radiological, Oncological and Pathological Sciences, Sapienza, University of Roma, Roma

³Dpt of Clinical Medicine, Roma

Idiopathic non-cirrhotic portal hypertension (INCPH) is a rare disease characterized by intrahepatic portal hypertension in absence of cirrhosis or other causes of liver disease and splanchnic venous thrombosis. Immunological disorders including Primary Antibody Defects (PADs) has been numbered among causes of INCPH. PADs include Common Variable Immunodeficiency (CVID), Hyper-IgM Syndrome (HIGM) and Good's Syndrome (GS). We assessed the prevalence of spleno-portal axis abnormalities in 150 PADs without known causes of liver diseases followed-up for a mean time of 20±14 years. Twenty-five percent had portal vein enlargement. In nine patients (6%) INCPH was diagnosed after 15±7 yrs since PADs diagnosis. All patients with INCPH had severe clinical and immunological phenotype, splenomegaly, recurrent gastrointestinal manifestations and high cholestasis enzymes. Liver biopsies showed liver sinusoids congestive dilatation, endothelialization, and micronodularity.

1) A HIGM woman with interstitial lung disease and recurrent gastrointestinal infections developed gastro-esophageal varices (GEV) at the age of 54. 2) A GS male with malabsorption, recurrent pneumonias and lymphadenopathy developed ascites and GEV at the age of 59. 3) A CVID male with sepsis, granulomatosis and lymphadenopathy developed GEV at the age of 25; he died at 57 years because of a fulminant pneumonia. 4) A CVID woman with lymphadenopathy and autoimmune enteropathy developed portal-systemic collaterals and ascites at the age of 32. 5) A CVID male with malabsorption, lymphadenopathy and autoimmune thrombocytopenia treated by splenectomy showed GEV at the age of 33. 6) GEV were described in a 63-years female with systemic granulomatosis and lymphadenopathy. 7-9) Portal-systemic shunts without GEV were observed in a 24-years CVID woman with eosinophilic pneumonia and uveitis, in a 56-years CVID woman with rheumatoid arthritis and in a 33-years CVID woman with recurrent pneumonia. During the follow-up none had variceal bleeding, liver failure nor portal vein thrombosis. No TIPS procedure was performed. Immune-abnormalities observed were: severe B-cell defects, increased numbers of exhausted CD21low B-cells and T-cell activation. In conclusion, in PADs INCPH had mild course but it was associated with poor PADs prognosis, due to concomitant disorders. Infections, inflammations, splenomegaly, increased blood venous flow, and lymphocyte abnormalities contribute to establish liver damage and INCPH.

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P5 - INNATE IMMUNITY AND INFLAMMATION

P4.7

DENDRITIC CELLS ACTIVATION IS ASSOCIATED WITH SUSTAINED VIROLOGICAL RESPONSE TO TELAPREVIR TREATMENT OF HCV-INFECTED PATIENTS

Alessandra Sacchi¹, Nicola Tumino¹, Federica Turchi¹, Giulia Refolo¹, Gian Maria Fimia¹, Fabiola Ciccocanti¹, Marzia Montalbano¹, Raffaella Lionetti¹, Chiara Taibi¹, Gianpiero D'Offizi¹, Rita Casetti¹, Veronica Bordini¹, Eleonora Cimini¹, Federico Martini¹, Chiara Agrati¹
¹INMI Lazzaro Spallanzani IRCCS, Roma

Purpose: First anti-Hepatitis C virus (HCV) treatments that include protease inhibitors (Direct Acting Antivirals, DAA) in conjunction with IFN- α and Ribavirin, increase the sustained virological response (SVR) up to 80% in patients infected with HCV genotype 1. However, the effects of triple therapies on immune system and in particular on Dendritic Cell (DC) compartment has not been yet investigated. In this study, we evaluated the effect of Telaprevir based triple therapy on DC frequency and functions, and the possible impact on treatment outcome.

Methods: Thirty-five HCV+ patients eligible for Telaprevir based therapy were enrolled, and circulating DC frequency, phenotype, and function were evaluated by flow-cytometry. The antiviral activity of pDC was evaluated on Rep60 cell line by measuring HCV RNA by RT-PCR.

Results: During triple regimen we observed a decrease of myeloid DC (mDC) frequency, that returned to baseline level when Telaprevir was stopped. In particular, mDC reduction occurred in patients who achieved the SVR. We also found that triple therapy induced the up-regulation of CD80 and CD86 on mDC from SVR, suggesting the induction of mDC maturation. This maturation is transient, since it dropped down at the end of therapy. Interestingly, only in SVR the treatment induced an improvement of IFN- α production by pDC after TLR7 stimulation, that was able to inhibit HCV replication in an in vitro assay system.

Conclusion: We showed that the achievement of SVR of HCV+ patients treated with Telaprevir-based triple therapy is associated with a transient activation of circulating mDC and pDC, suggesting a role of DC subsets in maintaining viral suppression. Further clinical studies using IFN-free regimens are needed to determine whether the DC activation during IFN-based therapy is due to the activity of IFN- α or to the decrease of viremia.

References

1. Schaefer et al. Gastroenterology 2012; 142: 134-5.
2. Mengshol JA et al. Gut 2009; 58: 964-73.
3. Sacchi A et al. Gut 2011; 60: 1161-2.

P5.2

CHARACTERIZATION OF INNATE IMMUNE CELLS FROM SPUTUM OF MTB EXPOSED SUBJECTS

Nicola Tumino¹, Federica Turchi¹, Delia Goletti¹, Gina Gualano¹, Fabrizio Palmieri¹, Rita Casetti¹, Veronica Bordini¹, Eleonora Cimini¹, Federico Martini¹, Chiara Agrati¹, Alessandra Sacchi¹
¹INMI Lazzaro Spallanzani IRCCS, Roma

Purpose: The events that induce the failure of protective immune response against M. tuberculosis (MTB) and the mechanisms used by MTB to force this failure are not completely understood. In particular, the role of innate cells against MTB in the site of infection, and in regulating T cell response remain to be elucidated. Aim of this work was to evaluate the phenotype and function of innate immune cells in sputum and in peripheral blood in individuals exposed to MTB and their association with the outcome of the infection.

Methods: We enrolled 22 household TB contact individuals. Induced sputum and peripheral blood were collected and the phenotype of monocytes, neutrophils and dendritic cells were analyzed by flow cytometry. Two individuals were Quantiferon negative (QFT-), 20 QFT+, and 1 subject, after 1 month from enrolment, had a sputum culture-positive for Mtb (MTB+).

Results: Monocytes of sputum from MTB+ individual had a lower expression of HLA-DR and TLR2 compared with QFT- and QFT+ subjects. Moreover, a higher expression of CD206 was observed in the MTB+ compared to the other groups, while the lowest expression was observed in QFT- subjects. Neutrophils from MTB+ subject express a higher level of CD63 compared with QFT- and QFT+ subjects.

In the peripheral blood the expression of CD206 was higher on monocytes from MTB+ subject than the other groups. A high frequency of CD16+/CD163+ anti-inflammatory monocytes was observed in MTB+ compared to QFT- and QFT+ subjects.

Conclusion: We performed a characterization of immune cells from sputum of MTB exposed subjects and found differences in the phenotype of monocytes and neutrophils associated with a different outcome of infection. Further analysis is mandatory in order to verify the possibility to use these markers as predictors of progression of infection

References

1. Orme IM, Robinson RT, Cooper AM. Nat Immunol 2015; 16: 57-63.
2. Leemans JC, Thepen T, Weijer S et al. J Infect Dis.2005; 191: 65-74.

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P5.3

ROLE OF MYD88-SIGNALING IN THE IMIQUIMOD-INDUCED MOUSE MODEL OF PSORIASIS: FOCUS ON INNATE MYELOID CELLS

Sara Costa¹, Dalila Bevilacqua¹, Olivia Marini¹, Silvia Lonardi², William Vermi², Marco A. Cassatella¹, Giampiero Girolomoni³, Patrizia Scapini¹

¹Department of Medicine, Division of General Pathology, University of Verona, Verona

²Department of Molecular and Translational Medicine, University of Brescia, Brescia

³Department of Medicine, Section of Dermatology and Venereology, University of Verona, Verona

Psoriasis is a chronic skin disease associated with deregulated activation of immune cells and keratinocytes [1].

Purpose: In this study, we utilized the imiquimod (IMQ)-induced mouse model of psoriasis to better dissect the contribution of hematopoietic and skin-resident stromal cells to psoriasis development.

Materials & Methods: Mice carrying either the total (Myd88^{-/-} mice) or the hematopoietic cell-specific (Myd88^{fl/fl}Vav-cre⁺ mice) or the monocyte/macrophage and neutrophil-specific (Myd88^{fl/fl}LysM-cre⁺ mice) deletion of MyD88 were utilized. Psoriasis development was induced by topical application IMQ-containing cream (AldaraTM) [2].

Results: By comparing disease development in Myd88^{fl/fl}Vav-cre⁺ mice with Myd88^{-/-} mice, we show that the progression of skin and systemic inflammation, as well as of epidermal thickening, were completely dependent on MyD88 expression in hematopoietic cells. However, both MyD88-deficient mouse strains developed some degree of epidermal thickening during the initial stages of IMQ-induced psoriasis even in the absence of hematopoietic cell activation and infiltration into the skin, suggesting a contribution of MyD88-independent mechanisms in skin-resident stromal cells. In addition, by utilizing Myd88^{fl/fl}LysM-cre⁺, we report that MyD88-signaling in monocytes and macrophages, but not in neutrophils, plays an important role in disease propagation and exacerbation by modulating their ability to sustain gδ T cell effector functions via IL-1b and IL-23 production.

Conclusions: Overall, these findings add new insights into the specific contribution of skin-resident stromal versus hematopoietic cells to disease initiation and progression in the IMQ-induced mouse model of psoriasis and uncover a novel pathogenic role for monocytes/macrophages to psoriasis development.

References

1. Perera GK et al. Annu Rev Pathol 2012; 7: 385-422.
2. van der Fits L et al. J Immunol 2009; 182: 5836-45.

P5.4

SKIN FUNGAL INFECTIONS: ROLE OF THE SITE AND ROUTE OF INFECTION ON T CELL ACTIVATION AND EFFICIENCY OF RECALL RESPONSES

Laura Gornati¹, Ivan Orlandi¹, Giulia Stamerra¹, Marina Vai¹, Ivan Zanoni², Francesca Granucci¹

¹Department of Biotechnology and Bioscience, University of Milano - Bicocca, Milano

²Harvard Medical School and Division of Gastroenterology, Boston Children's Hospital, Boston - USA

Purpose: The goal of our study is to deepen the role of immunological memory in the context of different skin fungal infections. Further, we aim to evaluate the influence of diverse sites and routes of infection on the establishment of memory T cells, unraveling the mechanisms underlying the faster clearance of pathogens in recall responses.

Methods: C57BL/6 mice are infected with blasts or hyphae of *Candida* (C.) *albicans*, subcutaneously (s.c.), transcutaneously (t.) or by scarification (sf.), on footpad or flank. Skin samples and skin-draining lymph nodes (D-LN) are collected at different time points post-infection for colony forming units (CFU) assay, histological staining and flow cytometry. Various immune pathways involved in the process will be evaluated using transgenic mice.

Results: The diverse models of infection display different routes of infiltration and kinetics of colonization of D-LN by *C. albicans*, as highlighted by CFU assay and histological sections. Preliminary data show the upregulation of T cell activation markers by D-LN CD4⁺ cells confirming the initiation of the adaptive response upon primary infection. Histological analysis of skin exhibits a reduced neutrophils recruitment but a more efficient clearance of pathogens in recall compared to primary responses.

Discussion: Both the primary adaptive response and the subsequent functionality of the immunological memory upon secondary immunizations are presumably influenced by the different routes of infection. The efficient removal of *C. albicans* in recall responses may be due to the effects of memory T cells on skin-resident or recruited cells that act at the site of re-infection.

Conclusions: The present work clarifies the effects of diverse skin fungal infections on T cell activation and memory T cell effector functions. We are currently focusing on the impacts that different routes of primary and secondary skin infections may have on recall responses whose fine mechanisms are still unexplored.

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P5.5

EXPRESSION OF PTX3 BY LYMPHATIC ENDOTHELIAL CELLS AND ROLE AS A GATE KEEPER AGAINST MICROBIAL DISSEMINATION

Marina Sironi¹, Andrea Doni¹, Sonia Valentino¹, Michela Calvi¹, Manuela Nebuloni², Fabio pasqualini¹, Fabio Pasqualini¹, Ana Rita Gomes¹, Annunciata Vecchi¹, Cecilia Garlanda¹, Barbara Bottazzi¹, Alberto Mantovani³

¹Humanitas Research Hospital, Rozzano

²Patology Unit, L Sacco Hospital, University of Milano, Milano

³Humanitas Research Hospital, Humanitas University, Rozzano

The long pentraxin PTX3 is a soluble pattern recognition molecule with multifunctional in vivo properties, ranging from the organization of a hyaluronic acid-rich extracellular matrix to female fertility and innate immune response to certain pathogens. PTX3 is produced by different immune or stromal cells in response to inflammatory signals or microbial recognition. Unexpectedly gene profiling revealed that both human and murine lymphatic endothelial cells (LECs) constitutively expressed PTX3. This observation prompted us to investigate the possible role of constitutively expressed PTX3 in the morphology and functionality of lymphatic vessels. Immunohistochemistry and confocal microscopy were used to analyze the presence and distribution of PTX3 in human and murine tissues. Morphometric analysis of murine lymphatics was performed after whole mounting in wild type and *ptx3*^{-/-} mice. Functional studies include fluid drainage and cell trafficking. Immunohistochemistry and confocal analysis confirmed the constitutive presence of PTX3 in human and murine normal tissues around lymphatic but not blood vessels. Morphometric analysis indicated that length, volume and cell area of lymphatic vessels in colon submucosa were increased in *ptx3*^{-/-} mice compared to wild type. *Ptx3*^{-/-} mice showed a reduced accumulation of Evans Blue dye in the draining popliteal lymph node at early time points (15-30 min.) after injection into the foot pad. Data in *ptx3*^{-/-} mice also indicated a reduced trafficking of dendritic cells to draining lymph nodes under inflammatory conditions. In a model of *Salmonella typhimurium* infection in *ptx3*^{-/-} mice, a higher dissemination of bacteria to the draining mesenteric lymph nodes was observed. Taken together these data indicate that PTX3 could play a non-redundant role in shaping and functionality of lymphatic vessels, acting as lymph node guardian preserving from bacterial diffusion.

P5.6

ROLE OF HYPOXIA AND THE TRIGGERING RECEPTOR EXPRESSED ON MYELOID CELLS IN HUMAN MACROPHAGE POLARIZATION

Federica Raggi¹, Simone Pelassa¹, Daniele Pierobon², Mirella Giovarelli², Federica Penco³, Luigi Varesio¹, Maria Carla Bosco¹

¹Laboratory of Molecular Biology, G. Gaslini Institute, Genova

²Department of Molecular Biotechnology and Health Sciences, University of Torino and Center for Experimental Research and Medical Studies (CERMS), AUO Città della Salute e della Scienza di Torino, Torino

³Department of Pediatrics, University of Genova and Pediatria II, G Gaslini Institute, Genova - Italy

Purpose: Macrophages (Mφ) are a major component of the leukocyte infiltrate at sites of inflammation and tumor growth. Mφ can undergo diverse forms of activation in response to environmental factors, polarizing into specialized functional subsets¹. A hallmark of the pathologic environment is represented by hypoxia¹. Little is known about the impact of the hypoxic environment on Mφ polarization. The objective of this study was to elucidate the effects of hypoxic conditions reflecting those occurring in vivo in diseased tissues on the ability of Mφ to polarize into classically activated (proinflammatory M1) and alternatively-activated (anti-inflammatory M2) subtypes.

Methods: Human peripheral blood monocytes were cultured for 6 days with M-CSF under normoxia (20% O₂) or hypoxia (1% O₂) and for additional 24 hr with LPS (for M1 polarization) or IL4 (for M2 polarization) and then phenotypically and functionally characterized.

Results: Hypoxia decreased Mφ expression of T cell costimulatory molecules and chemokine homing receptors and production of proinflammatory Th1-priming cytokines typical of M1 cells, while promoting the acquisition of M2 phenotypic and secretory features. Expression of the triggering receptor expressed on myeloid cells (TREM)-1² was induced in Mφ and its engagement imparted a proinflammatory M1-skewed phenotype to M2-polarized Mφ. Mφ infiltrating the inflamed hypoxic joints of children with Juvenile Idiopathic Arthritis² express TREM-1 and are predominantly polarized towards a M1 proinflammatory phenotype.

Discussion: We demonstrated that hypoxia exerts M2-polarizing effects on Mφ and identified TREM-1 as a marker of hypoxic Mφ and an inducer of M2 to M1 reprogramming under hypoxic conditions

Conclusions: These results highlight the fine regulatory control exerted by the hypoxic environment on Mφ polarization and point to a role of TREM-1 in JIA pathogenesis.

References

1. Bosco MC et al. Immunobiol 2008; 213: 733.
2. Bosco MC et al. Blood 2011; 117: 2625.

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P5.7

SYNERGISTIC ACTION OF PENTRAXIN 3 TOWARD MYELOPEROXIDASE-MEDIATED BACTERIA KILLING

Kenji Daigo¹, Diego Morone¹, Sonia Valentino¹, Marina Sironi¹,
Francesca Petroni¹, Andrea Doni¹, Antonio Inforzato¹,
Barbara Bottazzi¹, Alberto Mantovani¹

¹IRCCS Humanitas Research Hospital, Rozzano (Milan)

Purpose: Pentraxin 3 (PTX3) is a soluble-pattern recognition molecule that plays non-redundant protective roles against infection through complement regulation and recognition and opsonisation of certain microbes. PTX3 comprises eight-identical protomers, each consisting of a conserved Cterminal pentraxinlike domain and an Nterminal domain unrelated to other proteins. We previously reported that PTX3 interacts with myeloperoxidase (MPO), which is the most abundant bactericidal enzyme in neutrophils. Both PTX3 and MPO have been reported as protective molecules against *Aspergillus fumigatus* infection. Aim of our study was to investigate the role of PTX3-MPO interaction in *A. fumigatus* conidia killing. .

Methods and Results: By characterizing structural features of PTX3-MPO interaction, we found that PTX3 N-terminal domain was responsible for MPO binding. Interestingly, MPO enzymatic activity was increased in the presence of PTX3, an effect recapitulated by the PTX3 N-terminal domain. However the enzymatic activity of MPO bound to conidia from *A. fumigatus* was greatly suppressed and it remained suppressed also in the presence of full-length PTX3, while, unexpectedly, PTX3 N-terminal domain enhanced conidia-bound MPO enzymatic activity. On the contrary, we observed that PTX3 enhanced conidia killing by MPO in solution, and this action was mainly exerted by PTX3 N-terminal domain. In addition, only PTX3 N-terminal domain enhanced conidia killing by conidia-bound MPO. Finally, preliminary data indicated that PTX3 can amplify the MPO-dependent conidia killing exerted by NETs from human neutrophils.

Discussion and Conclusions: Our results show that PTX3, through its interaction with MPO, can amplify MPO-mediated conidia killing mainly through the N-terminal domain. This could represent a novel antimicrobial mechanism of PTX3. Further studies will be necessary to define the impact of our observations on the protective effects exerted by PTX3 against microbes.

P5.8

FUNCTIONAL ROLE OF FCEPSILONRI/IGE/ANTIGEN COMPLEXES EXPOSED BY MURINE MAST CELL DERIVED EXOSOMES

Mario Lecce¹, Rosa Molfetta¹, Linda Quatrini¹, Beatrice Zitti¹,
Alessandra Zingoni¹, Laura Masuelli², Giulio Caracciolo¹,
Angela Santoni³, Rossella Paolini³

¹Department of Molecular Medicine, ²Department of Experimental Medicine, "Sapienza" University of Roma, Roma

³Department of Molecular Medicine, "Sapienza" University of Roma - Institute Pasteur – Fondazione Cenci Bolognetti, "Sapienza" University of Roma, Roma

Purpose: Mast cells (MCs) are key effectors in allergic diseases: after activation by the high affinity receptor for IgE (FceRI), they release several pro-inflammatory factors including preformed mediators, eicosanoids, and various cytokines. Moreover, murine and human mast cells can also release exosomes [1], nanosized vesicles of endocytic origin that might act as intercellular communication vehicles. The aim of this study is the phenotypical and functional characterization of exosomes produced by unstimulated and IgE-stimulated MCs.

Methods: Exosomes were purified from the supernatant of Bone Marrow derived MCs (BMMCs) and of RBL-2H3 cell line by a series of ultracentrifugations and characterized by the combined use of electron microscopy, Dynamic Light Scattering and western blotting. The contribution of endosomal adaptors in exosome production was analysed by siRNA-mediated specific knock-down. The presence of FceRI/IgE complexes was evaluated by western blotting combined with FACS analysis of exosome-coated beads. MC degranulation was measured by b-hexosaminidase release assay, and exosome uptake was analysed by confocal microscopy and flow cytometry.

Results: We characterized MC-derived exosomes and demonstrated their endosomal origin. Next, we found that FceRI engagement increases the amount of exosomes with respect to constitutive-released vesicles, and that only exosomes released upon antigen (Ag) stimulation display both surface IgE and Ag. We have also demonstrated that vesicles exposing FceRI/IgE/Ag complexes are efficiently captured by sensitized MC and are able to induce their degranulation.

Conclusions: We demonstrate that upon MC stimulation by multivalent Ag, the FceRI/IgE/Ag internalized complexes can be released in exosomes. Those nanoparticles are easily uptaken by sensitized MCs and induce their activation, thus representing a potential mechanism of allergic reaction amplification.

Reference

1. Wilson B. *Frontiers in Immunology* 2012.

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P5.9

BIOASSAY TO ASSESS THE EFFECT OF ENVIRONMENTAL DOSES OF BISPHENOL-A ON HUMAN IMMUNE- COMPETENT CELLS

Alessandra Camarca¹, Stefania Picascia², Rossella Valentino³,
Carmen Gianfrani²

¹Institute of Food Sciences, ISA-CNR, Avellino

²Institute of Protein Biochemistry, CNR, Napoli

³Department of Public Health, Federico II University of Napoli,
Napoli

Purpose: Bisphenol-A (BPA) is one of most spread chemical contaminant with estrogenic activity. Recent studies suggest that may have a role in the increased incidence of inflammatory and allergic diseases. To address the effect of BPA on human immune system we set-up cellular bioassays with peripheral blood mononuclear cells (PBMCs), and monocyte-derived dendritic cells (mDCs). In particular, BPA was assayed at doses consistent with concentrations found in biological samples.

Methods: PBMCs from adult healthy donors were incubated for 48hrs in presence or absence of BPA (0.1 and 1nM) and stimulated or not with PHA or anti-CD3/anti-CD28 antibodies. The effect of BPA was assessed by evaluating the cell proliferation and cytokine production (IFN- γ , IL-4, IL-10 and IL-13). The differentiation/maturation status was evaluated in mDCs cultures generated in presence or absence of 1nM BPA.

Results: Under BPA exposure, PBMCs proliferation significantly increased, especially at 1nM concentration, whilst the production of both IL-10 and IL-13 resulted inhibited, mainly in mitogen-stimulated cells. By contrast, no significant effect was found on IFN- γ and IL-4 production. Finally, BPA increased the percentage of mDC expressing CD1a and concomitantly induced a reduction of HLA-DR and CD86 expression.

Discussion: The increase in proliferation and the decrease in IL-10 production by PBMC, as well as the up-regulation of CD1a on dendritic cells, indicate a pro-inflammatory effect of BPA on human immune cells. In addition, the decreased IL-13 production can be particularly important in the gut immune defenses.

Conclusion: Low doses of BPA affect the function of immune-competent cells, thus supporting its role in the development of immune diseases. In addition, PBMC and mDC short-term cultures can be a valid tool to address the immune-altering effects, in spite of toxicological effects, of food and environmental contaminants.

References

1. Rogers JA et al. 2013.
2. Kharrazian D. 2014.

P5.10

PRE-ECLAMPSIA IS ASSOCIATED WITH DEFECTIVE PRODUCTION OF C1Q BY INVASIVE TROPHOBLAST

Chiara Agostinis¹, Fleur Bossi¹, Oriano Radillo¹, Giuseppe Ricci¹, Tamara Stampalija¹, Leila Lo Bello¹,
Romana Videgar², Leonardo Amadio¹, Francesco Tedesco²,
Roberta Bulla²

¹Institute for Maternal and Child Health, IRCCS Burlo Garofolo,
Trieste

²Department of Life Sciences, University of Trieste, Trieste

Purpose: We have previously demonstrated that C1q, the first component of the classic complement cascade, is involved in the placentation process acting as a molecular bridge between endovascular trophoblast and decidual endothelial cell¹ and promoting trophoblast interstitial invasion². We observed also a deficient trophoblast invasion in implantation sites of C1q^{-/-} mice if compared to WT animals; so we hypothesized that C1q could had a role in the onset of pre-eclampsia, a multisystem syndrome characterized by a defect of placentation.

Methods: Placental mRNA derived from 7 pre-eclamptic (PE) patients and 6 healthy matched controls were analyzed by qPCR for C1q expression. PE sections were stained for C1q and cytokeratin 7 to identify trophoblast. The expression of MMP12 by on freshly isolated trophoblast cells adhering to C1q, FN or poly-L-Lysine was detected by qPCR and immunofluorescence.

Results: C1q expression was found to be significantly lower in PE placentae compared to healthy women. Histological evidences on PE decidual sections showed that trophoblast cells surrounding non-remodelled spiral artery do not express C1q in comparison to non pathological placentae. In vitro studies on trophoblast cells demonstrated that the expression of MMP-12, a marker of vascular remodelling³, is upregulated in response to C1q interaction.

Discussion: The defective staining of C1q by PE perivascular trophoblast seems to be directly related to absence of vascular remodelling. The upregulation of MMP-12 expression by trophoblast cells in response to C1q indicated a functional role of C1q in trophoblast vascular remodelling.

Conclusions: Collectively, these data support the pivotal role played by C1q in placental development. The importance of this component at the placental level is evidenced by its involvement in pregnancy disorders such as pre-eclampsia, characterized by poor trophoblast invasion.

References

1. Bulla et al. Mol Immunol 2008.
2. Agostinis et al. JRI 2010.
3. Harris et al. 2010.

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P5.11

GM-CSF AND IL-3 MODULATE HUMAN MONOCYTE TNF- α PRODUCTION AND RENEWAL IN IN VITRO MODELS OF TRAINED IMMUNITY

Raffaella Iannone¹, Francesco Borriello¹, Sarah Di Somma¹, Stefania Loffredo¹, Eloise Scamardella¹, Maria Rosaria Galdiero¹, Mariantonia Braile¹, Pasquale Esposito¹, Gilda Varricchi¹, Francescopaolo Granata¹, Giuseppe Portella¹, Gianni Marone¹

¹Department of Translational Medical Sciences, Napoli

Purpose: GM-CSF and IL-3 are hematopoietic cytokines that exert a significant control on monocyte and macrophage effector functions (1, 2, 3). We sought to investigate the mechanisms to which GM-CSF and IL-3 modulate LPS-mediated activation of human CD14⁺ monocytes taking into account the new concept of trained immunity.

Results: We demonstrate that GM-CSF and IL-3 priming enhances TNF- α production upon subsequent LPS stimulation (short-term model of trained immunity) in a p38- and SIRT2-dependent manner without increasing TNF primary transcript levels, supporting a post-transcriptional regulation of TNF- α in primed monocytes. GM-CSF and IL-3 priming followed by 6 days of resting also results in increased TNF- α production upon LPS stimulation (long-term model of trained immunity). In this case, GM-CSF and IL-3 priming induces a c-Myc-dependent monocyte renewal and increase in cell number that is in turn responsible for heightened TNF- α production.

Conclusions: Our results provide insights to understand the biology of monocytes in health and disease conditions and also extend our knowledge of the cellular and molecular mechanisms of trained immunity.

References

1. Croxford AL, Lanzinger M, Hartmann, FJ et al. The Cytokine GM-CSF Drives the Inflammatory Signature of CCR2⁺ Monocytes and Licenses Autoimmunity. *Immunity* 2015; 43: 50214. doi: 10.1016/j.immuni.2015.08.010.
2. Dabritz J, Weinhage T, Varga G et al. Reprogramming of monocytes by GM-CSF contributes to regulatory immune functions during intestinal inflammation. *J Immunol* 2015; 194: 2424-38. doi: 10.4049/jimmunol.1401482.
3. Mathias B, Szpila BE, Moore FA, Efron PA, Moldawer LL. A Review of GM-CSF Therapy in Sepsis. *Medicine (Baltimore)* 2015; 94: e2044. doi: 10.1097/MD.0000000000002044.

P5.12

GENETIC VARIATION IN AUTOPHAGY-RELATED GENES INFLUENCES THE RISK AND PHENOTYPE OF BURULI ULCER

Rita Silva-Gomes¹, Carlos Capela², Ange Dodji Dossou³, Ghislain Emmanuel Sopoh³, Michel Makoutode⁴, João Filipe Menino², Alexandra Gabriel Fraga², Cristina Cunha², Agostinho Carvalho², Fernando Rodrigues², Jorge Pedrosa²

¹Graduate Program in Areas of Basic and Applied Biology (GABBA), ICBAS, Porto - Portugal

²Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga - Portugal

³Buruli Ulcer Treatment Center of Allada, Allada - Benin

⁴Regional Institute for Public Health, Ouidah - Benin

Buruli Ulcer (BU) is a severe necrotizing human skin disease caused by *Mycobacterium ulcerans*. Clinical presentation is a sum of diverse pathogenic hits subjected to critical immune-regulatory mechanisms. Among them, autophagy has been demonstrated as a cellular process of critical importance. Since microtubules and dynein are affected by mycolactone, the pathogenic exotoxin produced by *M. ulcerans*, cytoskeleton-related changes might potentially impair the autophagic process and impact the risk and progression of infection.

Purpose: Genetic variants in the autophagy-related genes NOD2 (nucleotide-binding oligomerization domain-containing 2), PARK2 (E3 ubiquitin-protein ligase parkin) and ATG16L1 (autophagy-related protein 16-1) have been associated with susceptibility to mycobacterial diseases. Here, we investigated their association with BU risk, its severe phenotypes and its progression to an ulcerative form.

Methods: Genetic variants were genotyped using KASPar chemistry in 208 BU patients (70.2% with an ulcerative form and 28% in severe WHO (World Health Organization) category 3 phenotype) and 300 healthy endemic controls.

Results: The rs1333955 SNP (single nucleotide polymorphism) in PARK2 was significantly associated with increased susceptibility to BU (Odds ratio (OR) = 1.43; p = 0.05). In addition, both the rs9302752 and rs2066842 SNPs in NOD2 gene significantly increased the predisposition of patients to develop category 3 (OR = 2.23; p = 0.02; and OR = 12.7; p = 0.03, respectively), whereas the rs2241880 SNP in ATG16L1 was found to significantly protect patients from presenting the ulcer phenotype (OR = 0.35; p = 0.02).

Conclusion: Our findings indicate that specific genetic variants in autophagy-related genes influence susceptibility to the development of BU and its progression to severe phenotypes. Thus, our results provide crucial insights into the role of autophagy in the pathogenesis of BU.

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P5.13

ASBESTOS FIBERS REDUCE THE EXPRESSION OF C1q IN MACROPHAGES

Leonardo Amadio¹, Alessandro Mangogna², Chiara Agostinis¹, Romana Videgar², Elisa Trevisan², Violetta Borelli², Bulla Roberta²

¹Institute for Maternal and Child Health, Istituto di Ricovero e Cura a Carattere Scientifico Burlo Garofolo, Trieste

²Department of Life Sciences, Trieste - Italy

Purpose: Malignant pleura mesothelioma (MPM) is an aggressive tumor associated with asbestos exposure [1]. Asbestos fibers (AF) cause genetic/cellular damages of the mesothelial cells and chronic inflammation which can lead to carcinogenesis. In this context macrophage (M ϕ) fulfill a key role in the regulation of the inflammatory process. M ϕ are the main producers of C1q. C1q, besides its role in initiating the complement cascade and its function as opsonin in the process of phagocytosis and apoptotic cells clearance, is known to possess immunosuppressive properties (2). Recently, C1q has been shown to induce M2-like polarization of M ϕ [3].

The aim of our research was to investigate the contribution of the C1q in the pathogenesis of mesothelioma.

Methods: To understand how AF exposure could alter the phenotypic profile of M ϕ and modified their C1q expression M1 and M2 and resting macrophages (RM) obtained from peripheral blood derived monocytes were treated with crocidolite for 72h and qPCR assays, immunofluorescence analysis and ELISA experiments were performed.

Results: AF treated M ϕ showed an upregulation of TNF- α and CD80 and to a lesser extent of IL-1b. The upregulation was detectable in RM, M1 and even M2 polarized M ϕ . Vice versa genes of the M2-like phenotypic profile as CD206 and IL-10 resulted to be downregulated. AF treated M ϕ showed a reduced expression and production of C1q.

Discussion: As expected, our results showed that AF have induced a switch of RM to an M1-like phenotypic profile and in M2 polarized cells have reduced the expression of CD206 and IL-10. AF have a relevant impact in the downregulation of C1q expression by M ϕ affecting their role of apoptotic cell clearance.

Conclusions: The reduced expression of C1q by Mj can be responsible to the local chronic inflammation and to an environment prone to a neoplastic development.

References

1. Sekido Y. Carcinogenesis 2013; 34: 1413-9.
2. Son M et al. Blood 2016; 128: 2218-28.
3. Bohlson SS et al. Front Immunol 2014; 5: 402.

P5.14

ROLE OF THE ATYPICAL RECEPTOR CCRL2 IN THE PATHOGENESIS OF RHEUMATOID ARTHRITIS

Annalisa Del Prete^{1,2}, Cristina Mazzon¹, Francesca Sozio², Daniela Bosisio², Luisa Gazzarelli², Laura Tiberio², Eugenio Scanziani³, Annunciata Vecchi¹, Carlo Laudanna⁴, Alberto Mantovani¹, Silvano Sozzani¹

¹Humanitas Clinical and Research Center, Rozzano (MI), Rozzano (MI)

²Dept Molecular and Translational Medicine, University of Brescia, Brescia - Italy

³Dept Veterinary Sciences and Public Health, University of Milano, Milano

⁴Dept Medicine, University of Verona, Verona

Purpose: CCRL2 is a seven transmembrane domain receptor that shares structural and functional similarities with the family of the Atypical Chemokine Receptors. CCRL2 is upregulated by inflammatory signals, does not induce any intracellular signals and is expressed by many leukocyte subsets¹. CCRL2 was shown to be one of the most upregulated genes in neutrophils from the synovial fluid of rheumatoid arthritis patients². Since the spatio-temporal cascade of events responsible for the recruitment of neutrophils in the inflamed joints has been defined, we investigated in vitro and in vivo the potential contribution of CCRL2 in experimental models of inflammatory arthritis.

Methods: Collagen-Induced Arthritis was induced in CCRL2-deficient and WT mice by intradermal injection of 100 μ g type II chicken collagen, Serum-Transfer Induced Arthritis by i.p. injection of 150 μ l serum obtained from K/BxN mice. Disease severity was monitored daily using a standard clinical scale. Histological analysis was performed to the inflamed joints. The recruitment of leukocytes was evaluated after i.p. administration of CXCL8 and LPS by flow cytometry.

Results: Our preliminary results show that the CCRL2 deficient mice have defective neutrophil recruitment and are protected in experimental arthritis. Moreover, the in vivo administration of an anti-CCRL2 mAb protected WT mice from the onset of inflammatory arthritis.

Discussion: The molecular mechanism underlying the observed phenotype seems related to the functional interaction of CCRL2 with the prototypical neutrophil receptor CXCR2.

Conclusion: These results propose a new role for CCRL2 and suggest an additional mechanism of action of atypical chemotactic receptors in the regulation of inflammation. Moreover, CCRL2 might represent a new potential pharmacological target in the control of rheumatoid arthritis.

References

1. Del Prete et al. Eur J Immunol 2013; 43: 1418.
2. Galligan et al. Arthritis Rheum 2004; 50: 1806-14.

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P5.15

MITOCHONDRIAL MONOAMINE OXIDASE-DERIVED REACTIVE OXYGEN SPECIES ARE INVOLVED IN HUMAN MACROPHAGE M2 DIFFERENTIATION AND ACTIVATION

Veronica Costiniti¹, Regina Tavano¹, Antonella Viola¹, Marcella Canton¹

¹Dept. Biomedical Sciences, Venetian Institute of Molecular Medicine, Padova

Purpose: Reactive oxygen species (ROS) are well known to be fundamental for macrophages to kill invasive microorganisms. Moreover, they have an important role in regulating signal transduction pathways, gene expression and differentiation. Besides NADPH oxidase, mitochondria are gaining increasing relevance as a source of ROS in macrophages, although the exact sites of formation are only partially elucidated. Monoamine oxidase (MAO) is a relevant source of H₂O₂ in mitochondria, generated by oxidative deamination of biogenic amines. Since this enzyme has been scarcely characterized in phagocytic cells, we aimed at clarifying whether it plays a role in the differentiation and activation of macrophages.

Results and Discussion: The protein levels of both the MAO isoforms, A and B, increased significantly during differentiation of human monocytes to macrophages. Moreover, both LPS and IL-4+IL-13 caused a significant increase of MAO-A expression in M2 subtype as compared to resting macrophages. Concerning the induction pathway, both SB202190, a p38MAPK inhibitor, and mithramycin, a Sp1 transcription factor inhibitor, decreased MAO-A expression, suggesting a role for the p38MAPK-Sp1 axis in MAO-A induction. We then used the MAO specific inhibitor pargyline to analyze the role of MAO signaling in macrophage responses. Pargyline affected the expression of the M2 marker CD163 and prevented ROS formation and ERK phosphorylation only in M2 macrophages.

Conclusions: Taken together, these results provide the novel evidence that MAO contributes to ROS formation in macrophages and plays a relevant role in M2 differentiation and activation.

P5.16

REGULATION OF THE EXPRESSION OF IL-1R8, A REGULATORY MEMBER OF THE INTERLEUKIN-1 RECEPTOR FAMILY

Domenico Supino¹, Martina Molgora¹, Andrea Ponzetta¹, Marialuisa Barbagallo¹, Elena Magrini¹, Kevin Berthenet¹, Francesca Gianni¹, Fatemeh Asgari¹, Chiara Perucchini¹, Sebastien Jaillon¹, Alberto Mantovani¹, Cecilia Garlanda¹

¹IRCCS - Humanitas Clinical and Research Center, Rozzano, Milano

Purpose: IL-1R8 is an atypical Interleukin-1 receptor (ILR) family member capable of triggering a "multifaceted" anti-inflammatory program by inhibiting ILR and Toll like receptor (TLR) signaling (1). In NK cells IL-1R8 has been shown to act as a novel checkpoint inhibitor in cancer and viral infections. The purpose of the study was to investigate the mechanisms involved in regulating IL-1R8 expression.

Methods: By RT-PCR and FACS analysis, we investigated pathways that modulate IL-1R8 gene expression and protein level. RNA-seq and ChIP-seq datasets have been analyzed to predict the molecular machineries involved in IL-1R8 regulation.

Results: M-CSF derived macrophages showed higher IL-1R8 gene expression and protein level compared to GM-CSF derived macrophages. Pro-inflammatory molecules involved in M1 macrophage polarization down-regulated IL-1R8 in M-CSF derived macrophages. Prostaglandin PGE₂, which is involved in cancer-associated immunosuppression, up-regulated IL-1R8 in macrophages. Pro-inflammatory cytokines involved in NK cell activation down-regulated IL-1R8 in NK cells, and PGE₂ counteracted this effect.

In silico analysis indicated the existence of putative IL-1R8 truncated forms. RT-PCR experiments demonstrated that macrophages expressed increased levels of the exons coding for the intracellular part of IL-1R8 after M1 polarization. Finally, the expression of novel truncated forms of IL-1R8 was confirmed by western blot.

Discussion: These results indicate that pro-inflammatory stimuli are directly implicated in IL-1R8 down-regulation in macrophages and NK cells, and in the induction of truncated forms of the protein with unknown biological role. In addition, the up-regulation of IL-1R8 by PGE₂ suggests that IL-1R8 is part of the immunosuppressive activity of PGE₂.

conclusions: Since IL-1R8 is a crucial regulator of inflammation, the characterization of the regulation of its transcription is a promising tool to control its activity as immunocheckpoint.

Reference

1. Molgora M et al. Front Immunol 2016; 7: 149.

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P5.17

EFFICIENCY OF INTERFERON ALPHA-2-B SUBCUTANEOUS PARASPINAL INJECTION IN REDUCING OF HERNIATED DISC TISSUE

Alexander Tkachev¹, Alina Smirnova²

¹Novouzenskaya 6B-16, Volgograd - Russia

²Department of Radiology, MIBS, Saint-Petersburg - Russia

Purpose: The goal of this study was to evaluate the MRI-controlled efficacy of interferon alpha-2-b subcutaneous paraspinal injection on a size of regression of herniated disc tissue (HD) in patients with failure of conservative treatment.

Methods and Materials: A total of 22 patients were involved in this study. The MRI confirmed average size of HD was 10.6 mm ($\pm$ 0.8). All patients have a previously failure of conservative treatments and have indication for surgery measures to remove the herniated tissue. The duration of study was 60 days. Patients receive interferon alpha-2-b subcutaneous paraspinal injection in herniated disc level area, every other day, during 30 days. In total 15 injections. The dosage of interferon was 3 million international units. All patients received anticonvulsants for pain relief on different dosages. VAS score was used to evaluate intensity of pain. MRI scans was made every 10 days during study for each patient. After 60 days, we evaluate the average size of herniated disc regression in all patients by compare MRI scans.

Results: In 4 patients, no significant changes were observed on MRI. In other 18 patients, we evaluate the significant herniated disc regression. The average size of regression was 5.7 mm ($\pm$ 0.6). Also, we observed the changes of MRI signals in group that can be a precursor of further changes.

Conclusion: Interferon alpha-2-b has exerted effects through the induction of numerous IFN-stimulated genes and an immunomodulatory effect on innate and adaptive immune responses, inflammation and possibly can accelerate the act of regression of HD that can be in some cases an alternative to surgery measures. This results call for further research focused on immunobiology of this process by means with MRI diagnostic.

P5.18

STAT3 AND STAT5 DRIVE THE PRO-ANGIOGENIC NK CELL POLARIZATION IN COLORECTAL CANCER PATIENTS

Antonino Bruno¹, Barbara Bassani¹, Davide Giuseppe D'Urso¹, Silvia Zanellato², Ilvana Aida³, Luigi Boni³, Lorenzo Mortara², Douglas Noonan², Adriana Albini¹

¹Science and Technology Pole (PST), IRCCS MultiMedica, Milano

²Department of Biotechnology and Life Sciences, ³Department of Surgical and Morphological Sciences, University of Insubria, Varese

Purpose: We previously reported that NK cells from Non Small Cell Lung Cancer (NSCLC) acquire the CD56^{bright}CD16-VEGF^{high}PIGF^{high}IL-8⁺IFN γ ^{low} phenotype [1, 2]. We investigated whether peripheral blood and tumor infiltrating NK cells isolated from patients with colorectal cancer (CRC) have a unique pro-angiogenic profile and investigated the mechanisms.

Methods: NK subset distribution and cytokine profiling were performed by multicolor flow cytometry, using peripheral blood and tissue samples from CRC patients, for surface antigen and cytokine production. Supernatants from FACS-sorted NK cells were used for secretomic profiling, using antibody membrane array or in functional in vitro angiogenesis assays. Western blot analysis was used to determine molecular pathways modulated in CRC TINK/TANK.

Results: CRC NK cells express the decidual NK markers CD9 and CD49a and induced endothelial cell proliferation, migration, adhesion and formation of capillary-like structures on Human Umbilical Vein Endothelial Cells (HUVEC) in vitro. Secretome and flow cytometry analysis on CRC peripheral blood NK cells showed up-regulation of several pro-angiogenic factors, such VEGF, Angiogenin, Angiopoietin-1, Timp1-2, MMP-9. Molecularly, we observed p-STAT3 and p-STAT5 up-regulation in CRC peripheral blood NK and inhibition by pyrazole reduced both pro-angiogenic factor production and formation of capillary-like structures in vitro.

Discussion: Our data demonstrate that NK cells from CRC patients are switched toward a pro-angiogenic/pro-tumor phenotype and function and that STAT5 and/or STAT3 are involved.

Conclusions: Inhibitors of STAT5 and/or STAT3 could be useful in restoring NK cell function in CRC patients.

References

1. Bruno A et al. J Natl Cancer Inst 2014; 106: dju200.
2. Bruno A et al. Neoplasia 2013; 15: 133-42.

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P5.19

PLEURAL EFFUSION NK CELLS FROM METASTATIC TUMORS DISPLAY PRO-ANGIOGENIC FEATURES AND PLEURAL EFFUSION FLUIDS BLOCK THEIR RESPONSE TO IL-2 TREATMENT

Silvia Zanellato¹, Barbara Bassani², Annalisa Bosi³, Alessandra Musco³, Davide Giuseppe D'Urso², Maria Cattoni⁴, Claudia Sampietro⁴, Andrea Imperatori⁴, Lorenzo Dominioni⁴, Antonino Bruno², Lorenzo Mortara³, Douglas Noonan³

¹Department of Biotechnology and Life Sciences, University of Insubria, Varese

²Scientific and Technology Pole, IRCCS MultiMedica, Milano

³Department of Biotechnology and Life Sciences, ⁴Department of Medicine and Surgery University of Insubria, Varese

Purpose: Natural killer (NK) cells are effector lymphocytes crucial in tumor recognition and eradication. However, NK cell activity is often impaired in cancer patients (1). We recently demonstrated that they acquire proangiogenic phenotype and function in NSCLC [1, 2].

Methods: Here we characterized NK cells from peripheral blood (PB) and pleural effusions (PE) of patients with either inflammatory diseases, primary or metastatic tumors using flow cytometry, cell culture and NK degranulation assays.

Results: The highest percentage of PE immature NK cell subset CD56^{bright}CD16⁻ is found in malignant (60%), then in primary tumor (40%) and finally in inflammatory conditions (35%). PE-NK cells from tumor patients' display increased expression of the decidua NK marker CD49a, enhancement of the activatory antigen CD69 and decreased levels of the CD57 maturation marker. Although all NK cells from PE display higher expression of VEGF in comparison to autologous and healthy PB-NK cells, only metastatic PE-NK cells were statistically different. The cytotoxic potential was impaired as determined by a degranulation assays using K562 cells as targets compared to autologous and healthy PB-NK cells. Degranulation assays showed that PE-NK cells efficiently responded to IL-2 stimulation in vitro, however, addition of TGF β or cell-free PE supernatants in the culture blocked the response to IL-2.

Discussion: The tumor pleural effusion microenvironment produces factors that promote a preferential recruitment or expansion of CD56^{bright}CD16⁻ NK cells, preventing their maturation and cytotoxic function.

Conclusions: These data suggest a relevant role for PE tumor microenvironment in shaping NK cell polarization and establishing an alternative NK cell activation status.

References

1. Bruno A et al. Orchestration of angiogenesis by immune cells. *Front Oncol* 2014; 4: 1-13.
2. Bruno A et al. The proangiogenic phenotype of natural killer cells in patients with non-small cell lung cancer. *Neoplasia* 2013; 15: 133-42.

P5.20

EXTRACELLULAR VESICLES DERIVED FROM LICENSED MESENCHYMAL STEM CELLS: A TUNABLE APPROACH TO REGULATE ANGIOGENESIS

Roberta Angioni¹, Annamaria Tolomeo², Stephanie Herkenne¹, Bianca Cali¹, Maurizio Muraca², Antonella Viola¹

¹Dipartimento di Scienze Biomediche, ²Department of Women's and Children's Health, University of Padova, Padova

Purpose: Angiogenesis is the process that leads to the formation of new blood vessels from a pre-existing vascular network, playing a key role in many physiological and pathological processes. Consequently, targeting angiogenesis represents a very interesting therapeutic approach.

We recently showed that Mesenchymal Stem Cells (MSCs) regulate the inflammation-associated angiogenesis, thus controlling the immune response (1). However, MSC based therapy remains far from a fully developed and safe clinical technology. Importantly, a standardized and unifying protocol addressing which source of MSC should be used and which is the best route of administration is still missing. Even the parameters of quality and safety of MSCs are not universally established. To overcome all these issues, we focused our attention on an alternative approach, exploiting products of MSCs instead of MSCs themselves, which may represent a cost-effective and safer approach. Thus, the major goal of this study is the development of a feasible and plastic therapy, based on MSCs, which can target different inflammatory conditions associated with an altered angiogenesis.

Methods: One of the best-characterized MSC-product is the MSC-derived-extracellular vesicles (MSC-EVs) (2). To investigate MSC-EVs effect we first isolated and concentrated vesicles from the conditioned medium of stimulated MSCs with pro-inflammatory cytokines. We analysed the effect of MSC-derived EVs both in vitro, by tube formation and scratch assay, and in vivo, by matrigel plug assay and retina neovascularization mouse model.

Results: We found that MSC derived Extracellular Vesicles (MSC-EVs) recapitulate the functions of MSC both in vitro and in vivo and we developed a protocol to generate MSC-derived EVs with strong anti-angiogenic properties (PCT/IB2016/057608). In addition to the TIMP1-dependent mechanism that we have already described (1), we found that MSC-derived EVs inhibit VEGF responses in endothelial cells and affect Tip cells differentiation.

Conclusions: We believe that our EVs may represent a highly effective tool to treat conditions characterized by pathological angiogenesis.

References

1. Zanotti et al. Mouse mesenchymal stem cells inhibit high endothelial cell activation and lymphocyte homing to lymph nodes by releasing TIMP-1. *Leukemia* 2016; 30: 1143-54; doi:10.1038/leu.2016.33
2. Biancone L et al. Therapeutic potential of mesenchymal stem cell-derived microvesicles. *Nephrol Dial Transplant* 2012; 27: 3037-42. doi: 10.1093/ndt/gfs168.

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P6 - MUCOSAL IMMUNITY

P6.1 IMMUNOGENICITY OF A BIVALENT ADJUVANTED GLYCOCONJUGATE VACCINE AGAINST SALMONELLA TYPHIMURIUM AND SALMONELLA ENTERITIDIS

Fabio Fiorino¹, Simona Rondini², Francesca Micoli², Luisa Lanzilao², Renzo Alfini², Francesca Mancini², Calman MacLennan³, Donata Medaglini¹

¹Laboratorio di Microbiologia Molecolare e Biotecnologia (LA.M.M.B.), Dipartimento di Biotecnologie Mediche, Università di Siena, Siena

²GSK Vaccines Institute for Global Health S.r.l. (formerly Novartis Vaccines Institute for Global Health S.r.l.), Siena

³Jenner Institute, Nuffield Department of Medicine, University of Oxford, Oxford - United Kingdom

Purpose: Aim of this study was to investigate the systemic and local immune responses induced by *S. Typhimurium* and *S. Enteritidis* monovalent and bivalent glycoconjugate vaccines adjuvanted with aluminium hydroxide (alum) only or in combination with CpG ODN1826 (CpG) in two mouse strains.

Methods: CB6F1 and C57BL/6 mice were subcutaneously immunized at week 0, 4 and 9 with monovalent *S. Typhimurium* (O:4,5-CRM₁₉₇) and *S. Enteritidis* (O:9-CRM₁₉₇) conjugate vaccines (1,2), or with bivalent (O:4,5-CRM₁₉₇ + O:9-CRM₁₉₇) conjugate formulations, unadjuvanted or adjuvanted with alum only, or with alum plus CpG. Anti-O:4,5 and anti-O:9 antibodies in serum, intestinal washes and feces, serum bactericidal activity, and cytokine production in restimulated splenocytes were analyzed.

Results: All conjugate vaccines elicited high levels of serum IgG against the respective O-antigens (OAg) with bactericidal activity in both CB6F1 and C57BL/6 mouse strains. The bivalent conjugated vaccine induced systemic production of antibodies against both *S. Typhimurium* and *S. Enteritidis* OAg. The presence of alum or alum+CpG adjuvants in vaccine formulations significantly increased the serum antigen-specific antibody production. The alum+CpG bivalent vaccine formulation triggered the highest systemic anti-OAg antibodies and also a significant increase in anti-OAg IgG in intestinal washes and fecal samples, with a positive correlation with serum levels. Bivalent conjugate vaccines were more efficient in stimulating IL-2 production in the spleen compared to groups vaccinated with unconjugated OAg.

Discussion: These data demonstrate the ability of monovalent and bivalent conjugate vaccines against *S. Typhimurium* and *S. Enteritidis* to induce local and systemic immune responses in different mouse strains.

Conclusion: The bivalent glycoconjugate formulation, especially when adjuvanted with alum+CpG, is a promising candidate vaccine against iNTS disease.

References

1. Lanzilao L et al. PLoS ONE 2015; 10: e0139847.
2. Rondini S et al. Infect Immun 2015; 83: 996-1007.

P6.2

REGULATION OF DNAM-1 FAMILY RECEPTORS AND THEIR LIGANDS IN PHYSIOLOGICAL AND PATHOLOGICAL GUT MUCOSA INFILTRATE AND EPITHELIUM T CELL POPULATIONS

Lavinia Franchitti¹, Simone Battella¹, Angela Santoni¹, Gabriella Palmieri²

¹Department of Molecular Medicine, ²Department of Experimental Medicine, Sapienza University of Roma, Roma

Purpose: DNAM-1 family co-receptors are expressed on T lymphocyte subsets and provide activating (DNAM-1) or inhibitory (TIGIT) signals that regulate T cell functions and proliferation. We previously found that the expression pattern of these co-receptors strikingly differs between circulating and mucosal T cell populations. Moreover, perturbed expression of DNAM-1/ligand system distinctly characterizes infiltrate and epithelial counterpart in the inflamed mucosa microenvironment of active Inflammatory Bowel Disease (IBD) pediatric patients. Here we analyzed the capability of polyclonal TCR-dependent stimulation or selected cytokines to modulate the expression of DNAM-1 family co-receptors and shared ligands (PVR and Nectin-2) on peripheral blood (PB) T cell subsets and HT-29 colon carcinoma-derived cell line.

Methods: Healthy donor PBMCs were stimulated with anti-CD3/CD28 mAbs (3d); PBMCs or HT-29 were treated with selected cytokines (24h). Receptor and ligand expression levels were evaluated by immunocytofluorometric analysis.

Results and Discussion: IL-2 family cytokines or TCR/CD28 stimulation increases the frequency of TIGIT⁺ T cells, suggesting that such stimuli may partially explain the higher frequency of TIGIT⁺ mucosal T cells, as compared to PB counterpart. Differently, DNAM-1 levels are increased by TGF- β , and decreased by IL-17A. The dysregulated abundance of these two cytokines in inflamed mucosa microenvironment could underlie the downregulated DNAM-1 expression on mucosal T cells from active IBD patients. Moreover, Nectin-2 expression on HT-29 cells was decreased by TGF- β and IL-10 anti-inflammatory cytokines, suggesting that the reduced amount of these factors may lead to the increased frequency of Nectin-2⁺ gut epithelial cells recorded in active IBD lesions.

Conclusion: Our data suggest that mucosal microenvironment factors shape the physiological expression pattern of DNAM-1 family co-receptor/ligand system and contribute to its alteration in IBD.

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P6.3

ROLE OF THE ATYPICAL CHEMOKINE RECEPTOR CCRL2 IN A MODEL OF INTESTINAL INFLAMMATION

Tiziana Schioppa¹, Annalisa Del Prete¹, Francesca Sozio¹,
Andrea Piontini², Laura Melocchi³, William Vermi³,
Stefania Vetrano², Silvano Sozzani¹

¹Department of Molecular and Translational Medicine,
University of Brescia and Humanitas Clinical and Research
Center, Rozzano (MI)

²Humanitas Clinical and Research Center, Rozzano (MI)

³Department of Molecular and Translational Medicine, Section
of Pathology, School of Medicine, University of Brescia, Brescia

Purpose: Intestinal bowel disease (IBD), comprising Crohn's disease and ulcerative colitis, is a chronic inflammatory disease. The main pathological feature of IBD involves a massive infiltration of immune cells, in particular neutrophils into the intestinal tissue. Chemokine and their receptors play an important role in the recruitment of leucocytes in the intestinal mucosa of IBD patients [1]. CCRL2 is a seven-domain transmembrane receptor that apparently does not activate any chemokine conventional signaling. This receptor is rapidly induced in many leucocytes, including neutrophils [2]. The aim of our work was to define the role of CCRL2 during an experimental model of inflammation-induced colitis (dextran sodium sulphate-DSS) by using CCRL2 deficient mice (KO).

Methods: WT and CCRL2 KO mice were treated with 2% DSS for 7 days and body weight, bleeding and stools consistency were monitored. On the day of termination, mice were euthanized and colon were collected for histological analysis. To evaluate the damage cause by DSS treatment, colon epithelial permeability was assessed with Evans blue dye.

Results: Our preliminary results show that the CCRL2 KO show exacerbated phenotype, including higher body loss, increased bleeding, shorter colon length and higher damage of colon epithelial barrier compared to WT animals.

Discussion: The results obtained support a protective role for CCRL2 in a model of IBD. The mechanisms responsible for the observed phenotype in CCRL2 KO is under investigation, but our preliminary data indicate a role for this receptor not only at the level of leukocyte compartment but also in the maintaining the integrity of the epithelial barrier.

Conclusion: The understanding of the role of CCRL2 in the recruitment of immune cells at colonic mucosa level or on the epithelial cells might provide new insights in the treatment of pathological intestinal inflammation.

References

1. Katoh H et al. Cancer Cell 2013.
2. Del Prete A et al. Eur J Immunol 2013.

P7 - NEUROIMMUNOLOGY

P7.1

ACTIVATION STATE AND FUNCTIONALITY OF DENDRITIC CELLS FROM PERIPHERAL BLOOD OF AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

Michela Rusconi¹, Francesca Gerardi², Andrea Lizio², Christian Lunetta², Ivan Zanoni³, Francesca Granucci¹

¹Departement of Biotechnology and Biosciences, University of
Milano Bicocca, Milano

²NEMO clinical center, Niguarda Hospital, Milano - Italy

³Boston's Children Hospital Division of Gastroenterology,
Harvard Medical School, Boston, MA - USA

Purpose: Chronic inflammation is important for neurodegeneration in Amyotrophic lateral sclerosis (ALS) with an increased spinal cord (SC) recruitment of peripheral proinflammatory monocytes, dendritic cells (DCs) and T cells. The aim of the present study was to examine circulating DCs in ALS patients in order to lay the basis to understand how these cells contribute to disease progression

Methods: DC numbers and their phenotype were investigated by cytofluorimetric analyses and the spontaneous or LPS-induced production of inflammatory cytokines was measured by ELISA.

Results: We found that ALS patients have much lower number of circulating DCs compared with healthy donors, and their DCs show an increased expression of the integrin CD62L. A subpopulation of ALS patients had a higher spontaneous and LPS-induced IL8 production. These patients also showed higher efficiency of CCL2 secretion. Moreover, we observed a significant inverse correlation between the time from onset to diagnosis and the Δ IL6 levels.

Discussion: The lower DC number and the higher expression of CD62L in ALS patients confirmed that DCs are actively recruited in the central nervous system (CNS). Although we could not define a correlation between the higher CCL2 and IL8 production and disease progression, high levels of CCL2 have been shown in the SC of SOD mice, and in some ALS patients, so DCs can be a source of CCL2 in the SC in a subpopulation of ALS patients.

Conclusion: DCs are one of the major cell subset recruited to the CNS at least in some ALS patients. Peripheral blood DC analyses can be useful to stratify patients in those that have a high inflammatory response versus those that do not show an altered inflammatory pathway. The high levels of CD62L expression by peripheral blood DCs suggest that this molecule could be a possible target for in vivo treatment.

References

1. Henkel JS et al. 2004
2. Zhang R et al. 2005.

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P7.2

EFFECT OF MIMETIC NERVE GROWTH FACTOR TRKA AGONIST MT2 NON PEPTIDE MOLECULE IN EAE AND CUPRIZONE MODEL

Antonio Sibilla¹, Elena Bonechi¹, Alessandra Aldinucci¹, Luca Massacesi¹, Federico Cozzolino², Clara Ballerini¹

¹NEUROFARBA, Firenze

²Dipartimento di Scienze Biomediche, Sperimentali e Cliniche (DSBSC), Firenze

Purpose: To study the effects of MT2, non-peptidic Nerve Growth Factor (NGF) mimetic, agonist of NGFR Tropomyosin receptor kinase TrkA and TrkB on Experimental Autoimmune Encephalomyelitis (EAE), animal model of multiple sclerosis (MS) and on toxic central nervous system demyelination (cuprizone). MT2 acts on neuronal cell death through the MAPK/ERK pathway (unpublished data), therefore our specific aim was to investigate if MT2 can counteract pathological events that occur in MOG₃₅₋₅₅ induced EAE [1] and demyelination occurring without inflammatory reaction.

Methods: EAE and cuprizone treatment as reported elsewhere [1, 2]; 150 µl of MT2 (1,33 mg/ml) was daily injected i.p. for a total of 9 days from 14th day post immunization and after 7 weeks of cuprizone; clinical score and spinal cord histopathology were determined. Cuprizone demyelination was evaluated means transmission electron microscope; in EAE ex vivo immune response was evaluated. Results. MT2 ameliorates EAE clinical scores ($p < 0.0001$) compared to controls in agreement with observed fewer infiltrates and areas of demyelination ($p < 0.0001$). Ex vivo isolated T cells showed increased production of IL10 ($p < 0.05$). On the other hand, MT2 treatment of toxic demyelination showed that the treatment worsens demyelination and partially delays remyelination.

Discussion: Our data suggest that MT2 ameliorates EAE as potent antiinflammatory agent, with no or even detrimental effect on pure degenerative mechanisms, when tissue damage is occurring without inflammatory reaction. This could be due to MT2 ability of binding TrkB, known to be involved in astrocyte detrimental reaction to nervous system damage [3]. NGF receptors are widely expressed in immune cells, therefore it will be of interest to investigate MT2, able to cross blood brain barrier, as effective agent in late MS phases, where inflammatory events are completely compartmentalized and difficult to treat.

References

- 1 Bonechi et al. 2016.
- 2 Gudi et al. 2014.
- 3 Colombo et al. 2012.

P7.3

DYSREGULATION OF REPRESSOR ELEMENT 1-SILENCING TRANSCRIPTION FACTOR IN A MOUSE MODEL OF MULTIPLE SCLEROSIS

Valentina Petrosino¹, Stefania Criscuolo², Federica Buffolo², Fabrizia Cesca², Nicole Kerlero de Rosbo¹, Fabio Benfenati², Antonio Uccelli¹

¹Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa, Genoa

²Center for Synaptic Neuroscience, Istituto Italiano di Tecnologia, Genoa

The Repressor Element-1 Silencing Transcription factor (REST) is a negative master regulator of neurogenesis and neuronal identity. While REST is quiescent in mature neurons, dysregulation of REST and its repercussion on the target genes have been implicated in several neurodegenerative disorders [1, 2].

Purpose: Our goal is to assess the role of REST in experimental autoimmune encephalomyelitis (EAE), a mouse model for multiple sclerosis, characterized by inflammation, demyelination and axonal loss.

Methods: Chronic EAE was induced in C57Bl/6J mice by immunization with myelin oligodendrocyte glycoprotein peptide. mRNA expression of REST and target genes was analyzed by RT-PCR on brain and spinal cord tissues.

Results: REST expression increased significantly in EAE mouse spinal cord 24 hours after disease onset, with concomitant downregulation of the voltage-dependent Na⁺ channel Nav1.2, confirming REST transcriptional repression and suggesting neuronal dysregulation at this early stage. Time course analysis confirmed overexpression of REST in both spinal cord and striatum during acute phase. However, while upregulation of REST correlated with downregulation of its target genes in the spinal cord, it was unexpectedly associated with upregulation of the same genes in the striatum. Because REST4, a REST splicing variant, has been shown to induce derepression of REST target genes [3], we monitored its expression which, interestingly, was increased at the early phase in the striatum, where we observed the upregulation of the target genes.

Conclusions: These data suggest that REST dysregulation also occurs in EAE, influencing the response of neural cells to pathological stimuli. Whether an unbalance in the expression of REST and REST4 affects the anomalous expression of REST in EAE at specific stages and in particular areas remains to be established.

References

1. Chong JA. REST: a mammalian silencer protein that restricts sodium channel gene expression to neurons. *Cell* 1995; 80: 947-57.
2. Lu T. REST and stress resistance in ageing and Alzheimer's disease. *Nature* 2014; 507: 448-54.
3. Shimojo M. Protein kinase A regulates cholinergic gene expression in PC12 cells: REST4 silences the silencing activity of neuron-restrictive silencer Factor/REST. *Mol Cell Biol* 1999; 19: 6788-95.

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P7.4

DIFFERENTIAL USE OF THE HYDROXYCARBOXYLIC ACID RECEPTOR-2 PATHWAYS TRIGGERED BY MONOMETHYL FUMARATE IN DIFFERENT CELLS

Benedetta Parodi¹, Nicole Kerlero de Rosbo¹, Antonio Uccelli¹

¹Neuroimmunology Unit, Department of Neuroscience (DINOGLI), University of Genoa, Genoa

Purpose: We have demonstrated that monomethyl fumarate (MMF), the anti-inflammatory bioactive metabolite of dimethyl fumarate (DMF), modulates microglia activation towards M2-like phenotype through a novel pathway triggered by MMF binding to hydroxycarboxylic acid receptor-2 (HCAR2) that leads to inhibition of NF- κ B via the AMPK/Sirt1 axis. Increasing evidence associates signaling through HCAR2 in macrophages and dendritic cells (DC) with an anti-inflammatory phenotype; MMF could therefore exert its effect also in these cells by activating the AMPK/Sirt1 axis. Since HCAR2 is also a receptor for butyrate (But), an anti-inflammatory commensal metabolite, we have speculated that intestinal side effects associated with DMF treatment might be related to MMF competition with But for HCAR2 binding, with MMF signaling in these cells operating through the prostaglandin D2/inflammatory pathway, whereas But, which blocks NF- κ B activation in colonic cells, would signal through AMPK/Sirt1. Our aim therefore is to define the use of HCAR2-triggered pathways in these different cell types relevant to DMF treatment.

Methods: Spleen DC were isolated through magnetic bead (anti-CD11c) affinity sorting. Gene expression and pathway activation were assessed by real time PCR and western blotting, respectively.

Results: MMF partially inhibited bone marrow-derived macrophage activation, reducing Nos2 expression without modulating that of other typical markers, suggesting that in these cells MMF does not signal through AMPK-Sirt1. Similarly, while MMF induced an anti-inflammatory phenotype in activated splenic DC, reducing the expression of Tnf, Il12 and Il23, it had no such effect on activated bone marrow-derived DC. Our preliminary results, demonstrating modulation of microglia activation by But along with an increase in phospho-AMPK, indicate that But could signal through the novel AMPK/Sirt1 pathway.

Conclusion: Altogether these data suggest that HCAR2 signaling through different pathways could be cell- and ligand-biased.

P7.5

A POSSIBLE ROLE FOR NERVE GLIAL ANTIGEN 2 IN DENDRITIC CELL ACTIVATION

Giovanni Ferrara¹, Sara Morando¹, Federico Ivaldi¹, Nicole Kerlero de Rosbo¹, Antonio Uccelli¹

¹Department of Neurology, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa, Genoa

We recently showed that experimental autoimmune encephalomyelitis (EAE) induced with myelin oligodendrocyte glycoprotein (MOG) in nerve/glia-antigen 2 (NG2) knock-out (NG2KO) mice results in a milder disease than in wild-type (WT) mice [1]. Upon recall T-cell responses to MOG NG2KO T cells were skewed towards a less inflammatory Th2-type response that was not due to an inherent defect of NG2KO T cells, and chimera experiments showed that regardless of their original phenotype, mice receiving NG2KO bone marrow developed milder EAE than those receiving WT bone marrow. We found that, in addition to macrophages, NG2 was also expressed in WT mice by most T cells and 40-50% dendritic cells (DC) and that the proportion of activated IL-12-expressing DC was significantly lower in NG2KO mice. Together with our observation that IL-12-expressing cell population in WT mice is smaller in CD11c+ NG2- cells than in CD11c+ NG2+ cells, these data suggested that NG2 could be involved in DC activation.

Aim: To define the role of NG2 in DC activation. **Methods:** DC derived from bone-marrow (BMDDC) using GM-CSF and sorted by flow cytometry using antibodies to CD45, CD11c and NG2 were stimulated overnight with or without LPS/IFN γ . IL-12 in supernatant was assessed by ELISA.

Results: To understand if NG2 is constitutive or induced upon activation, NG2- and NG2+ sorted BMDDC were analyzed by cytometry. While the percent of NG2+ cells did not change upon stimulation (90%), the percent of NG2+ cells in the sorted NG2- cells after overnight stimulation increased from 38% to 75%, with a concomitant decrease in NG2- cells from 54% to 19%, suggesting that, NG2 expression is induced in DC upon activation. Induction of NG2 was accompanied with an increase in IL-12 expression.

Conclusion: Our data suggest that NG2 could play a role in DC activation and could therefore be an important target of inflammation.

Reference

1. Ferrara G, Errede M, Girolamo F, et al. NG2, a common denominator for neuroinflammation, blood-brain barrier alteration, and oligodendrocyte precursor response in EAE, plays a role in dendritic cell activation. *Acta Neuropathol* 2016; 132: 23-42.

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P7.6

EXPLORING THE ROLE OF MICRORNAS IN THE INTRA-THYMIC PATHOGENESIS OF MYASTHENIA GRAVIS

Claudia Barzago¹, Paola Cavalcante¹, Stefania Marcuzzo¹, Silvia Bonanno¹, Teresio Motta², Carlo Antozzi¹, Fulvio Baggi¹, Pia Bernasconi¹, Renato Mantegazza¹

¹Neurology IV, Neurological Institute Carlo Besta, Milan

²Department of Pathological Anatomy, Bologni Hospital, Serrate

Purpose: Myasthenia gravis (MG) is a B-cell mediated autoimmune disorder of the neuromuscular junction. Thymus is generally accepted as being a key organ in which the auto-sensitisation process takes place in acetylcholine receptor-positive MG patients (AChR-MG)¹. However, the exact intra-thymic mechanisms involved in autoimmunity development and perpetuation in AChR-MG patients are not completely known. In our previous study, we found a dysregulated microRNA (miRNA) signature in peripheral blood cells of these patients. In particular, miR-612, miR-3651, and miR-3654 were upregulated in AChR-MG samples compared to healthy controls². Here, we aim to investigate the possible role of these miRNAs – and others known to be dysregulated in MG peripheral blood – in the intra-thymic MG pathogenesis.

Methods: We analysed the expression levels of selected miRNAs, and their putative target genes, in AChR-MG (i.e. follicular and diffuse hyperplastic) and normal control thymuses by real-time PCR.

Results: We obtained data indicative of altered miRNA expression in the thymus of AChR-MG patients compared to controls.

Discussion: Our overall findings suggest a contribution of miRNAs to the immunological alterations responsible for autoimmunity initiation or perpetuation in AChR-MG thymus.

Conclusions: Our studies may contribute to gain knowledge on the molecular mechanisms associated with AChR-MG pathogenesis, paving the way towards possible miRNA-based therapeutic interventions.

References

1. Berrih-Aknin S, Le Panse R. Myasthenia gravis: a comprehensive review of immune dysregulation and etiological mechanisms. *J Autoimmun* 2014; 52: 90-100.
2. Barzago C et al. A novel infection- and inflammation-associated molecular signature in peripheral blood of myasthenia gravis patients. *Immunobiology* 2016; 221: 1227-36.

P7.7

NEUROINFLAMMATION AND PERIPHERAL GLUTAMATE UPTAKE IN MULTIPLE SCLEROSIS: A CORRELATION STUDY

Maira Gironi¹, Chiarapaola Zoia², Marina Saresella³, Guido Cavaletti², Mario Clerici⁴, Giancarlo Comi⁵, Carlo Ferrarese², Roberto Furlan¹

¹Unit of Neuroimmunology, Dep of Neurology, Univ. Vita e Salute, OSR, Milan

²Dept. of Neuroscience, University of Milano-Bicocca, Monza

³IRCCS, S Maria Nascente Don Gnocchi Foundation, Milan

⁴IRCCS, Don Gnocchi Foundation, Milan

⁵INSPE, San Raffaele Scientific Institute, Milan

Excitotoxicity mediated by glutamate overload is a pathogenic mechanism shared by different neurodegenerative diseases, Multiple Sclerosis (MS) among others. High-affinity-sodium-dependent-Excitatory-Amino-Acid-Transporters (EAATs) clear about 90% of glutamate released at synaptic cleft, stain neurons and glial cells and are also present in platelets (PLT). Increasing evidences sustain PLT as reliable biomarkers in neurodegenerative diseases.

Purpose: 1) Measuring EAAT in PLT of MS patients with different clinical courses, and healthy controls (HC). 2) Investigating any relationship between immune peripheral-blood-mononuclear-cells (PBMCs) and EAAT platelet function.

Methods: We measured EAAT function in platelets of 78 MS patients and 61 HC. Primary progressive (PP = 17), secondary progressive (SP=18), relapsing remitting (RR = 29) and benign (BB=14) patients were enrolled. Sodium/energy-dependent-glutamate-uptake was studied in PLT measuring tritiated-Glutamate by beta-counter. The expression of different transcription factors and cytokines production involved in the differentiation of Th1, Th2, Th17, and Treg have been analysed in PBMCs and correlated to PLT glutamate uptake.

Results: Reduced glutamate uptake values were found in MS patients compared to HC (19.56 vs 27.81, $p = 0.01$) as well as significant differences were found across MS clinical courses (HC = 27.81; PP = 19.2; SP = 12.21; RR = 17.62; BB = 33.46 glutamate pmoles/mg prot/30 min; $p < 0.0001$). After performing Bonferroni correction, strong inverse correlations were found between EAAT function and Th1 cells ($r = -0.59-0.64$; $p < 0.002$).

Conclusions: Strong evidences suggest a bidirectional influence between inflammatory cells and glutamate transporter activity. The finding of an inverse correlation between EAAT activity and Th1 cells confirm a relationship between EAAT impairment and high percentage of inflammatory cells.

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P7.8

AN OPTIMIZED PROTOCOL OF DIFFERENTIAL CENTRIFUGATION ISOLATES DISTINCT MICROVESICLE SUBPOPULATIONS FROM MYELOID CELLS

Annamaria Nigro¹, Alessandro Romano¹, Annamaria Finardi¹, Marzia M. Ferraro², Antonio Gaballo², Daniela E. Manno³, Angelo Quattrini¹, Roberto Furlan¹

¹Division of Neuroscience, Institute of Experimental Neurology, San Raffaele Scientific Institute, Milano

²CNR NANOTEC, Nanotechnology Institute, Lecce

³Department of Mathematics and Physics "E. De Giorgi", University of Salento, Lecce

Purpose: Extracellular vesicles (EVs) are heterogeneous populations of cell-derived vesicles involved in many physiological/pathological processes with major potential as biomarkers. It is well recognized that cells essentially secrete two EV subtypes: a larger size class (microvesicles, MVs; 100-1000 nm) and a smaller size class (exosomes; 50-150 nm). In particular, MVs originate by direct outward budding of the cell membrane. Their isolation protocols are still elusive, and so far, differential centrifugation remains the most commonly used isolation method. However, various parameters in MV isolation procedure, such as the use of different rotor types, can influence their recovery. Here, we provide a comparative evaluation of the utilization of different rotor types during differential centrifugation protocol, such as the swinging bucket and fixed angle rotors, for the yield and purity of isolated vesicles.

Methods: We determine recovery efficiency, morphology and dimension of myeloid-derived MVs by flow cytometry, electron and atomic force microscopy. RNA and protein quantification was used to characterize MVs.

Results: Our results demonstrate that the application of a fixed angle rotor during the first centrifugation step harvests greater yield of purified MVs. Moreover, the rewashing of the usually discarded first pellet increases microvesicle recovery, and allows isolation of a different subpopulation of microvesicles showing distinct morphological and molecular characteristics. Interestingly, the results identify a different profile in terms of RNA/protein ratio with distinct RNA yields between the two microvesicle subpopulations.

Conclusions: Overall, our results point to demonstrate that isolation method significantly influences MV yield and quality. Thus, we propose an optimized protocol for the purification and characterization of the heterogeneous group of myeloid-derived MVs.

P7.9

ANNEXIN A1 (ANXA1): POTENTIAL REGULATOR OF ADAPTIVE IMMUNE RESPONSE IN MULTIPLE SCLEROSIS (MS)

Alessandra Colamatteo¹, Elisa Maggioli², Silvana Cassano¹, Mario Galgani¹, Sara Bruzzaniti¹, Giuseppe Matarese³, Egle Solito², Veronica De Rosa¹

¹Istituto per l'Endocrinologia e l'Oncologia Sperimentale, Consiglio Nazionale delle Ricerche (IEOS-CNR), Napoli

²William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London - United Kingdom

³Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli "Federico II", Napoli

Purpose: ANXA1 is a glucocorticoid-induced anti-inflammatory molecule that affects innate immunity by limiting neutrophils extravasation and blocking monocytes migration via $\alpha 4 \beta 1$ integrin [1]. It regulates blood brain barrier (BBB) integrity in brain microvascular endothelial cells and its expression is selectively lost in BBB of MS subjects [2]. Our aim was to investigate whether ANXA1 could have also a role in the modulation of adaptive immunity, both in healthy and naive-to-treatment MS subjects.

Methods: We measured ANXA1 expression in different T cell subsets: CD4⁺CD25⁺Foxp3⁺ regulatory T (Treg), CD4⁺CD25⁻Foxp3⁻ conventional T (Tconv) and CD4⁺ROR γ t⁺ (Th17) cells, by flow cytometry. Moreover, we evaluated whether ANXA1 could be differentially expressed in MS individuals and how its plasma levels, measured through ELISA assay, correlated with disease severity and progression. Finally, we also investigated the migratory capacity of Treg and Tconv cells from MS and healthy subjects, using a transmigration assay with human brain microvascular endothelial cells.

Results: ANXA1 plasma levels in MS subjects inversely correlated with disease severity (EDSS and relapses number). Its expression was significantly lower in Treg, Tconv and Th17 cells from MS compared to healthy subjects. This finding correlated with an higher degree of adhesion and migration of both Treg and Tconv cells in vitro.

Discussion: Guided by the working hypothesis that ANXA1 impairment may contribute to an unbalanced Th1-Th17/Treg cell ratio in MS patients, together with the in vivo data on the ability of this protein to restore BBB functionality, our idea is that ANXA1 is a key molecule at the boundary between BBB permeability and immune function.

Conclusion: Understanding the molecular mechanism accounting for the reduced ANXA1 expression should provide relevant informations on the key events leading to MS onset and progression, thus unveiling novel potential therapeutic settings for the disease.

References

1. Solito E et al. J. Immunol. 2000.
2. Cristante E et al. PNAS 2013.

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P7.10

ATAXIC SENSORY NEURONOPATHY AS SENTINEL SYMPTOM OF PRIMARY SJÖGREN'S SYNDROME: A CASE REPORT

Irene Tempesta¹, Giuliana Conca¹, Elena Luciannatelli¹, Antonio Iaffaldano¹, Teresa Francavilla¹, Marco Guido¹, Giuseppe Libro¹, Carla Tortorella¹, Isabella Laura Simone¹

¹Department of Basic Medical Sciences, Neurosciences and Sense Organs, University of Bari "Aldo Moro", Bari

Introduction: Primary Sjögren's syndrome (pSS) is an autoimmune disorder characterized by lymphocytic infiltration of exocrine glands leading to a sicca syndrome. Ataxic sensory-neuronopathy (ASN) is a complication of pSS characterized by severe impairment of deep sensation.

Case Report: A 61 year-old woman came to our attention referring a 10 years history of dysesthesias of the right hand, difficulties of the right upper limb movements and progressive gait abnormalities limiting daily life activities. One year before hospitalization she received pSS diagnosis and began treatment with methotrexate. At the admission to our Neurological Department she was able to walk only with bilateral support because of severe sensitive ataxia. Neurological examination revealed dysarthria, apallesthesia, dysmetria, pseudoathetotic movements of both arms, dystonic attitude of the fingers and areflexia. Brain and spinal cord MRI were normal. Electrophysiological study revealed a sensory-motor axonal neuropathy. Cerebrospinal fluid (CSF) analysis showed normal cells and protein content and 11 CSF restricted oligoclonal bands. Laboratory tests revealed ANA positivity with nuclear speckled pattern (titer 1:160), anti-SSA 185,8 UA/mL (< 10). A diagnosis of sensory-motor axonal neuropathy pSS related was performed and she was treated with periodic intravenous immunoglobulins (IVIg 0.4 gr/Kg die for 5 days) with clear improvement of both gait disturbance and athetoid movements.

Conclusions: This case report confirm that severe ASN could be a sentinel symptom of pSS. Early diagnosis is crucial since IVIg has been reported to be an effective treatment whereas other immunotherapies failed to impact neurological deterioration.

References

1. Fauchais AL et al. Central and peripheral neurological complications of primary Sjögren's syndrome. *Presse Med* 2012; 41: e485-93.
2. Takahashi Y et al. Benefit of IVIG for long-standing ataxic sensory neuronopathy with Sjögren's syndrome. *Neurology* 2003; 60: 503-5.

P7.12

IL-27, BUT NOT IL-35, INHIBITS NEUROINFLAMMATION THROUGH MODULATING GM-CSF EXPRESSION

Annamaria Finardi¹, Hélène Descamps², Federico Colombo³, Chiara Maiorino¹, Francesca Ruffini², Marco Patrone⁴, Massimo Degano⁴, Gianvito Martino², Luca Muzio², Burkhard Becher⁵, Giacomo Casella³

¹Clinical Neuroimmunology Unit, Department of Neuroscience, Institute for Experimental Neurology, ²Neuroimmunology Unit, ⁴Biocrystallography Unit, Department of Immunology, Transplantation and Infectious Diseases, Department of Neuroscience, Institute for Experimental Neurology, San Raffaele Scientific Institute, Milano

³Università Vita e Salute San Raffaele, Milano

⁵Inflammation Research, Institute of Experimental Immunology, University of Zurich, Zurich - Switzerland

Background: IL-27 and IL-35 are heterodimeric cytokines, members of the IL-12 family and considered to have regulatory properties. Their role during neuroinflammation had been investigated using mutant mice devoid of either one of the subunits or lacking components of their receptors, resulting in conflicting results.

Objective: we sought to understand the therapeutic potential of IL-27 and IL-35 vehiculated by gene therapy in neuroinflammation.

Methods: We constructed lentiviral vectors expressing IL-27 and IL-35 from a single polypeptide chain, and we validated in vitro their biological activity. We injected these IL-27 and IL-35-expressing lentiviral vectors into mice affected by experimental neuroinflammation (EAE), and performed clinical, neuropathological and immunological analyses.

Results: Both cytokines interfere with neuroinflammation, but only IL-27 significantly inhibits disease development, both clinically and neuropathologically. IL-27 protects from autoimmune inflammation by inhibiting granulocyte macrophages colony-stimulating factor (GM-CSF) expression in CD4⁺ T cells and by inducing program death-ligand 1 (PD-L1) expression in both CNS-resident and CNS-infiltrating myeloid cells.

Conclusion: We demonstrate here that IL-27 and IL-35 hold therapeutic potential during neuroinflammation and that IL-27 inhibits GM-CSF and induces PD-L1 in vivo. We propose IL-27 as a candidate therapy for neuroinflammatory disorders such as multiple sclerosis.

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P7.13

MONOGENIC METABOLIC DISEASES AS MODELS TO STUDY COMPLEX AUTOIMMUNE DISORDERS LIKE MULTIPLE SCLEROSIS

Fortunata Carbone¹, Daniela Melis², Teresa Micillo¹, Claudia La Rocca¹, Veronica De Rosa¹, Sara Bruzzaniti¹, Roberta Lanzillo³, Vincenzo Brescia Morra, Giuseppe Matarese⁴

¹Laboratorio di Immunologia, Istituto di Endocrinologia e Oncologia Sperimentale, Consiglio Nazionale delle Ricerche (IEOS-CNR), Napoli

²Dipartimento di Scienze Mediche Traslazionali, Sezione di Pediatria, ⁵Dipartimento di Neuroscienze, Scienze Riproduttive ed Odontostomatologiche, ⁴Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli "Federico II", Napoli

Purpose: Peripheral immune tolerance is maintained at least in part by regulatory T (Treg) cells. It has been recently shown that specific metabolic pathways are involved in the control of Treg cells generation and function as glycolysis drives the conversion of Tconv cells into induced Treg (iTreg) cells. The relationship between metabolism and autoimmunity is also supported by epidemiological observations showing that patients affected by glycogen storage disease type-1b (GSD-1b), a monogenic disorder characterized by defective glucose/glycogen metabolism, have an increased risk for developing autoimmune disorders. We aimed at investigating firstly the metabolic alteration occurring in patients affected by multiple sclerosis (MS) and in parallel we recapitulated these defects in the immune system of GSD-1b where glucose utilization is impaired and an altered immune tolerance is present.

Methods: We analysed the extracellular acidification rate (ECAR) and the oxygen consumption rate (OCR), indicators of aerobic glycolysis and oxidative phosphorylation respectively in T cells from MS and GSD-1b patients, respectively. In addition, in both disorders we also evaluated the efficiency of induction of Foxp3 upon activation of Tconv by western blot analyses and the suppressive function of peripheral Treg cells.

Results: We observed an impaired glycolysis during Tconv activation in MS, associated with a reduced suppressive capacity of iTreg cells. Similarly to MS subjects, in GSD-1b subjects we also found reduced engagement of glycolysis in T cells together with reduced suppressive function of Treg cells and reduced induction of Foxp3.

Discussion: Our data suggest that impaired glycolytic metabolism associates with reduced induction of Foxp3, impaired suppressive function, and it represents a common feature of an altered immune tolerance.

Conclusions: These data provide molecular evidence on the link among Treg cell function, autoimmunity and impaired glucose metabolism. GSD-1b could be used as a model for the study of how immunometabolism regulates immune tolerance in multifactorial human autoimmune diseases such as MS.

P7.14

MONOCYTE MICROVESICLES AS POSSIBLE TARGET FOR FINGOLIMOD ACTION IN MULTIPLE SCLEROSIS

Antonella Amoruso¹, Maria Blonda¹, Roberta Grasso¹, Valeria Di Francescantonio¹, Carlo Avolio¹

¹Department of Medical and Surgical Sciences, University of Foggia, Foggia

Purpose: Microvesicles (MV), a way of intercellular communication, have been implicated in several biological processes, including neuroinflammation. MVs shedding is a process induced by the ATP receptor P2X7 expressed on immune cells especially of the myeloid lineage and it is associated with activation of acid sphingomyelinase (ASMase) and with inflammatory cytokine IL-1b release. Our purpose was to evaluate how Fingolimod may affect MVs production from monocytes of Multiple Sclerosis (MS) patients and ASMase activity.

Methods: Thirty-six MS patients were enrolled. Nineteen of them started assuming Fingolimod. Purified monocytes from PBMCs were isolated from venous blood samples after 12 months of treatment. MVs were evaluated by fluorimetry. ASMase activity was determined using Amplex Red sphingomyelinase assay. P2X7R, IL-1b and ASMase expression were quantified by qRT-PCR. Fifteen healthy donors (HD) were also recruited.

Results: Fingolimod reduced MV production. The amount of shed vesicles was higher in MS than in HDs ($p < 0.001$) and Fingolimod treated patients ($p < 0.0001$) only in unstimulated monocytes. Upon BzATP stimulation, MV shedding increased only in HDs ($p < 0.001$) and Fingolimod ($p < 0.05$) but not in untreated patients. P2X7R expression was higher in MS, even if not statistically significant and treatment did not reduce such expression. Treatment instead was able to reduce IL-1b expression. Fingolimod decreased ASMase activity both in unstimulated ($p < 0.05$) and in stimulated monocytes ($p < 0.001$) if compared with MS without altering its expression.

Discussion/Conclusions: These data suggest that monocytes from MS patients produce vesicles in a higher amount than controls and Fingolimod treated patients. We possibly provided evidence for a novel effect of Fingolimod, suggesting that treatment reduced MV production by inhibiting ASMase activity, without modifying P2X7R expression.

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P7.15

SOLATION AND CHARACTERIZATION OF AUTOREACTIVE CD4+ T CELLS IN NARCOLEPTIC PATIENTS

Eric Armentani¹, Daniela Latorre¹, Ulf Kallweit², Mathilde Perez¹, Federico Mele¹, Francesco Zellini³, Mauro Manconi³, Ramin Khatami⁴, Mehdi Tafti⁵, Claudio Bassetti², Federica Sallusto¹

¹Center of Medical Immunology, Institute for Research in Biomedicine, Università della Svizzera Italiana, Bellinzona - Switzerland

²Neurology Department, University Hospital, Bern - Switzerland

³Ospedale Regionale di Lugano (EOC), Lugano - Switzerland

⁴Center for Sleep Research and Sleep Medicine, Clinic Barmelweid, Barmelweid - Switzerland

⁵Center for Investigation and Research in Sleep (CIRS), Lausanne University Hospital (CHUV), Lausanne - Switzerland

Purpose: Narcolepsy (NC) is a rare chronic neurological sleep-wake disorder that is caused by the selective loss of neuronal cells of the posterior hypothalamus that produce the neuropeptide hypocretin (HCRT). Accumulating lines of evidence support the notion that NC is an immune-mediated disorder that manifests in genetically predisposed individuals; genome-wide association studies showed a strong association with HLA-II DQB1*06:02, antibodies against the influenza nucleoprotein were shown to cross-react with HCRT receptor 2 in H1N1 virus infected patients and increased number of NC cases in Europe and Asia after Pandemrix[®] vaccination campaign has been reported. The aim of the present study is to identify and isolate from NC patients, auto-reactive T cells, in order to study their functional properties, TCR repertoire and expansion and localization in vivo.

Methods: Antigenic stimulation, T cell cloning, and TCR deep sequencing have been combined to characterize the T cell response in HLA-II DQB1*06:02 positive NC patients. T cells were isolated from blood and cerebrospinal fluid (CSF) and expanded polyclonally to generate T cell libraries that were interrogated for antigen-specific reactivity. Next-generation TCR sequencing (NGS) was performed on total T cells from blood and CSF in order to gather information on NC patients' TCR repertoire.

Results: Auto-reactive CD4+ T cell clones could be isolated from the blood of NC patients, but not in HLA-II DQB1*06:02- positive controls. The clones use different TCR α/β and recognize several regions of the HCRT. Moreover additional autoantigens, selectively expressed by HCRT+ hypothalamic neurons, can be recognized by CD4+ T cells of NC patients. NGS identified "public" clonotypes present in the blood of NC patients, some of which are highly expanded.

Conclusions and Discussions: Collectively, these data provide the first evidence of autoreactive CD4+ T cell clones directed against HCRT and other neuronal antigens in the blood of NC patients that may play a role in the disease, findings strong evidences of the immune basis of NC.

References

1. Nohynek H et al. PLoS One 2012.
 2. Partinen M. PLoS One 2012.
 3. Miller E et al. BMJ 2013.
 4. Mahlios J et al. Curr Opin Neurobiol 2013.
 5. Ahmed SS et al. Sci Transl Med 2015.
 6. Bernard-Valnet R et al. PNAS 2016.
 7. Hartmann FJ et al. J Exp Med 2016.
- (619) Neuroimmunology

P7.16

A MODEL OF THE BLOOD BRAIN BARRIER TO INVESTIGATE IMMUNE TRAFFICKING IN NEUROLOGICAL DISORDERS

Eliana Lauranzano¹, Raffaella Molteni², Fabia Filippello¹, Ruggero Pardi³, Michela Matteoli¹

¹Humanitas Clinical and Research Center, IRCCS, Rozzano, Milano

²San Raffaele Scientific Institute, Milano

³Vita-Salute San Raffaele University School of Medicine, Milano

Purpose: The blood-brain barrier (BBB) is a highly specialized barrier separating the brain parenchyma from the vascular compartment. It plays a crucial role in regulating the entry of blood-borne molecules and preserving homeostasis within brain microenvironment. Progressive BBB breakdown and loss of solute barrier are associated with a diverse range of neuroinflammatory and neurodegenerative conditions.

Methods: We are developing an in vitro BBB model consisting of a contact co-culture of brain endothelial cells (EC) growing as a monolayer on top of a matrix-coated permeable membrane (vascular BBB side), and primary astrocytes cultured on the opposite side (CNS BBB side) with astrocyte endfeet taking contact with EC.

Results and Discussion: We monitored transendothelial electrical resistance (TEER) as the main indicator of the functional formation of the barrier. Barrier integrity was confirmed by the formation of tight junctions between adjacent EC, by immunofluorescence for claudin-5 and ZO-1, connexin-43, with restricted paracellular diffusion of water-soluble substances. Permeability assays using the impermeable dye LY and the hydrophilic dye NaF confirmed low (MW $\leq$ 550Da) solute passive transport. The ability of the barrier to prevent protein extravasation was confirmed by measurements of permeability to 10kDa-Dextran. Inserts with fully differentiated BBB were placed in a microfluidic platform to assess transmigration of immune cells. Real-time 4D tracking of cells under flow conditions revealed the initial rolling and adhesion of leukocytes to the endothelium, followed by subsequent diapedesis across the BBB and interstitial migration.

Conclusions: This microfluidic platform provides a new and versatile tool to investigate in vitro the stepwise process of circulating immune cell extravasation across the BBB and to test novel therapeutics targeting various steps of the process.

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P7.17 MULTIPLE SCLEROSIS TREATMENTS AFFECT MONOCYTE DERIVED MICROVESICLE PRODUCTION

Maria Blonda¹

¹University of Foggia, Foggia -

Microvesicles (MVs) are small vesicles which bud directly from the cell plasma membrane and are released into the extracellular environment by a variety of cells, especially immune cells and those of the myeloid lineage, upon stimulation with ATP on its cognate receptor P2X7, in physiological conditions and in higher amount upon cellular activation. Increased release of MVs has been described as being associated with the acute or active phase of several neurological disorders, therefore their role as biomarkers of tissue damage is assuming relevance and is especially helpful to examine districts which are not accessible. To date, the role of MVs in Multiple Sclerosis is generally still very poorly understood and remains little investigated. We aimed to study the release of MVs in P2X7R-stimulated peripheral blood monocytes from MS patients and healthy donors (HDs) and to see how current DMDs with a different mechanism of action may affect such a production. We also aimed to study the treatment effect on M1 and M2 monocyte subtypes and on the inflammasome components. Twenty untreated relapsing-remitting MS patients and 20 healthy donors (HDs) were selected in order to obtain purified monocytes from total peripheral venous blood. A subgroup of 91 FN band and a subgroup of 5 Teriflunomide treated MS patients were evaluated before and after 2, 6 and 12 months of treatment. Another subgroup of 6 MS patients assuming Fingolimod, after switching from a first line therapy, was included in the study and analyzed only at 12 months of treatment. Spectrophotometric quantification of MVs was performed and M1 and M2 markers other than P2X7R and inflammasome component expression were quantified by qRT-PCR. We observed that monocytes from MS patients produced vesicles per se in higher amounts than controls whereas BzATP, mimicking inflammatory stimulation, induces a strong vesicle release only in HDs compared to non inflammatory conditions. All treatments reduced vesicle production and IL-1B expression, but only Teriflunomide was associated with a down-regulation of P2X7R. Therapies modulated both M1 and M2 monocyte subtypes. This was strongly evident for Teriflunomide, probably because of its immunosuppressive properties. Our results, suggesting new molecular targets for drugs currently used in MS, may potentially provide useful evidence in MS therapeutics in the near future.

P7.18 DYSREGULATED IRF-1 PATHWAY IN PERIPHERAL B CELLS OF MS PATIENTS

Roberta Reniè¹, Marco Salvetti¹, V. Annibali¹, R. Umeton¹, M. Severa², M.P. Etna², S. Giglio³, S. Romano¹, M. Ferraldeschi¹, M.C. Buscarinu¹, A. Vecchione², A. Annese¹, C. Policano¹, R. Mechelli¹, G. Mattei¹, D.F. Angelini³, E.M. Coccia⁴, G. Ristori¹

¹Department of Neurology, University la Sapienza, Roma

²Division of Pathology, Department of Clinical and Molecular Medicine, Sant'Andrea Hospital, Faculty of Medicine and Psychology, Roma

³Neuroimmunology Unit, Fondazione Santa Lucia, Roma

⁴Department of Infectious, Parasitic and Immune-mediated Disease, Istituto Superiore di Sanità, Roma

Background

There is evidence of a role for B cells in multiple sclerosis (MS).

Objective: A stringent experimental/statistical setting allowed to obtain a whole transcriptome (coding and non-coding RNAs) analysis on peripheral B cells from relapsing-remitting patients.

Methods: We performed microarray studies on 10 therapy-free patients with stable (clinical-MRI) disease, and 10 age- and sex-matched controls. A software package plus a bioinformatics pipeline based on open-source software was implemented for data analysis. Data validation was performed through RT-PCR in an extended study population, and with functional experiments focused on the relevant dysfunctional pathway.

Results: Among 6 differentially expressed genes in MS, two down-regulated transcripts (IRF1 and CXCL10), belonging to the same pathway, were validated by RT-PCR in a total of 26 patients and 21 matched controls. Performing an analysis between dysregulated transcripts and miRs, we found that both IRF1 and CXCL10 had a potential seeding sequence for hsa-miR-424 (one of the miRs up-regulated in MS patients). Being CXCL10 transcription directly regulated by IRF1, we focused on hsa-miR-424-IRF1 mRNA 3'-UTR interaction, and confirmed its functional effect at the protein level.

Discussion: Our results highlight a dysregulated IRF-1/CXCL10 axis in B cells from patients with relapsing-remitting MS, suggesting a B cell dysfunctional, pro-survival status in MS.

Conclusion: Our findings point to a dysregulated IRF-1/CXCL10 axis in MS.

References

1. Disanto G et al. Neurology 2012; 78: 823-32.
2. Ratzer R et al. Mult Scler 2013; 19: 1841.
3. Sievers C et al. Altered microRNA expression in B lymphocytes in multiple sclerosis: towards a better understanding of treatment effects. Clin Immunol 2012; 144: 70-9.

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POSTER SESSION II

P8 - CHRONIC LYMPHOCYTIC LEUKEMIA

P8.1 PROGNOSTIC AND PREDICTIVE ACTIVITY OF IGVH MUTATION IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

Andrea Visentin¹, Monica Facco¹, Veronica Martini¹, Federica Frezzato¹, Silvia Imbergamo¹, Filippo Severin¹, Flavia Raggia¹, Valentin Trimarco¹, Leonardo Martinello¹, Edoardo Scomazzon¹, Francesco Piazza¹, Gianpietro Semenzato¹, Livio Trentin¹

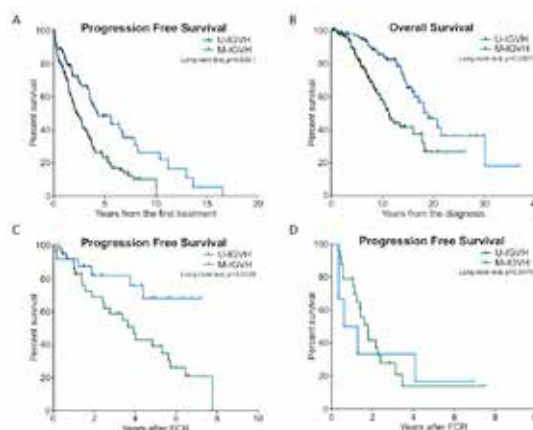
¹Hematology and Clinical Immunology unit, Department of Medicine, University of Padova, Padova

Purpose: Prognostic and predictive markers are commonly used to identify patients with increased risk of progression and death, the former, or early relapse after treatment, the latter. The aim of this study was to investigate the prognostic and predictive role of IGVH mutation in patients with chronic Lymphocytic Leukemia (CLL).

Methods: Among the 816 patients followed at the Hematology Unit of Padova from 1989, 527 had productive rearrangement of the B-cell receptor. IGVH mutational status was tested at CLL diagnosis. Homology > 98% of IGHV gene from the germline sequence identify unmutated cases (U-IGVH), otherwise they were considered mutated (M-IGVH). Survival curves were compared with log-rank test and plotted using Kaplan-Meier method.

Results: The prognostic activity was assessed in the whole cohort of 527 patients. The median progression free survivals (PFS) were 2.9 and 15.7 years for U and M-IGVH patients ($p < 0.0001$). U-IGVH subjects also had a shorter overall survival (OS) compared to M-IGVH (11.5 vs 30.1 years, $p < 0.0001$). To evaluate the predictive strength of IGVH mutational status we analyzed 256 patients who required treatment during the follow-up. U-IGVH subjects had almost 2-fold increase risk of relapse and death after first-line therapy than M-IGVH (hazard ratio 1.9 and 2.4, respectively, $p < 0.0001$). In particular, we focused on 64 treatment-naïve (TN) and 30 relapsed or refractory (R/R) patients who received fludarabine-cyclophosphamide-rituximab (FCR) chemoimmunotherapy. The median PFS was 3.9 for U-IGVH but not reached for M-IGVH patients ($p = 0.0328$) among TN subgroup, while there was not difference in the R/R subgroup (1.8 and 0.9 years, $p = 0.8479$, for U-IGVH and M-IGVH, respectively).

Discussion and Conclusions: Accordingly to data from the literature IGVH was able to identify patients with early progression and with an increase risk of death. In addition we shed light on the predictive strength of IGHV mutation on all treated patients in particular among those who received FCR as first line therapy, but not in the R/R setting.



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P8.2

THE E3 UBIQUITIN LIGASE C-CBL IN THE REGULATION OF BCR SIGNALLING IN CHRONIC LYMPHOCYTIC LEUKEMIA

Veronica Martini¹, Rosa Molfetta², Filippo Severin¹, Valentina Trimarco¹, Federica Frezzato¹, Flavia Raggi¹, Leonardo Martinello¹, Andrea Visentin¹, Monica Facco¹, Gianpietro Semenzato¹, Rossella Paolini², Livio Trentin¹

¹Department of Medicine, Hematology and Clinical Immunology Branch, University School of Medicine, Padova

²Department of Molecular Medicine, University of La Sapienza, Roma

Purpose: In normal B cells, the E3 ubiquitin ligase Cbl (c-Casitas B-lineage lymphoma) is involved in the ubiquitin-dependent Lyn degradation and in the down-regulation of BCR signaling [1]. We reported that in Chronic Lymphocytic Leukemia (CLL) Lyn is over-expressed and is in an active conformation as integral component of an aberrant cytosolic multiprotein complex [2]. Here, we investigated the expression and the role of c-Cbl in CLL B cells.

Methods: Blood samples were collected from 30 CLL patients and 15 controls. Untouched peripheral blood B cells were purified using the RosetteSep isolation kit for human B cells. We characterized c-Cbl total protein level and Y700 phosphorylation by Western blotting. We performed a co-immunoprecipitation assay to evaluate the interaction between c-Cbl and Lyn in CLL B cells at steady state and after IgM stimulus.

Results / Discussion: We demonstrated that in CLL B lymphocytes c-Cbl is overexpressed with respect to normal B cells and did not co-immunoprecipitate with Lyn neither after BCR trigger. We showed that in CLL cells the phosphorylation on Cbl Y700 increased after 5' of IgM stimulus, highlighting its potential involvement in BCR signaling. Since in myeloid malignancies mutations at linker/RING finger regions of Cbl are responsible for loss of ligase activity [3], we investigated c-Cbl mutational status in these patients but we did not find any mutations.

Conclusions: In CLL cells c-Cbl is overexpressed with respect to normal B cells, and upon BCR engagement it undergoes Y700 phosphorylation. However, c-Cbl is unable to stably interact with Lyn suggesting an altered c-Cbl function that contribute to affect cell homeostasis.

References

1. Shao Y. J Biol Chem 2004; 279: 43646-53.
2. Trentin L. Blood 2008; 112: 4665-74;
3. Bunda S. Oncogene 2015; 34: 789-97.

P8.3

NOCODAZOLE TREATMENT LEADS TO NEOPLASTIC CELL APOPTOSIS IN THE CHRONIC LYMPHOCYTIC LEUKEMIA MOUSE MODEL Eu-TCL1

Filippo Severin¹, Veronica Martini¹, Michele Azzolini², Luigi Leanza², Federica Frezzato¹, Valentina Trimarco¹, Flavia Raggi¹, Leonardo Martinello¹, Andrea Visentin¹, Monica Facco¹, Mario Zoratti², Ildiko Szabó²,

Gianpietro Semenzato¹, Livio Trentin¹

¹Department of Medicine, Hematology and Clinical Immunology Branch, Padova

²Department of Biology, Padova

Purpose: Microtubule network of cell cytoskeleton plays a crucial role in the vital functions of malignant Chronic Lymphocytic Leukemia (CLL) B lymphocytes, including mitosis, motility and cell-cell contact, and, for this reason, they became an important target in cancer therapies. We demonstrated that the microtubule inhibitor nocodazole was highly specific in inducing apoptosis of ex vivo leukemic cells from CLL patients¹. With this as background we were aimed to evaluate nocodazole effectiveness and toxicity in a CLL murine mouse model Em-TCL1 characterized by high expression of T-Cell Leukemia 1 (TCL1) protein in B cells leading to the development of a CLL-like lymphoproliferative disease².

Methods: At the age of 13-18 months, cells from Em-TCL1 peripheral blood sample were stained with antibodies specific for murine CD5, CD19, CD3, CD4 and CD8 and evaluated by flow cytometry. Once assessed the presence of disease, 5 mice were treated with nocodazole 10ng/Kg diluted in a solution of dimethyl sulfoxide (DMSO) +10% of polyethylene glycol 350 (PEG350) and 5 control mice with only DMSO+10% of PEG350 (5 days/week for 4 weeks).

Results: After 4 weeks of therapy, we observed an improvement of treated mice in term of appearance, posture and weight gain) with respect to controls. We also demonstrated a decrease in total lymphocyte percentage after nocodazole administration and a higher reduction of pathological B cells in spleen and bone marrow of treated, with respect to untreated, mice.

Conclusions: The high selectivity of nocodazole and its capability to induce apoptosis in CLL B cells also in the Em-TCL1 mouse model, may suggest the use of this inhibitor for designing new therapeutic strategies.

Evaluation of further cases could allow to confirm the pro-apoptotic action on neoplastic B cells and the increase of cytotoxic T cells due to nocodazole treatment.

References

1. Frezzato F et al. Br J Haematol 2014; 165: 659-72.
2. Bichi R et al. PNAS 2002; 99: 6955-60.

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P8.4

HSP70 ROLE IN SURVIVAL OF NEOPLASTIC B LYMPHOCYTES FROM CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

Flavia Raggi¹, Federica Frezzato¹, Veronica Martini¹, Valentina Trimarco¹, Filippo Severin¹, Leonardo Martinello¹, Edoardo Scomazzon¹, Andrea Visentin¹, Monica Facco¹, Francesco Piazza¹, Gianpietro Semenzato¹, Livio Trentin¹

¹Department of Medicine, Hematology and Clinical Immunology Branch, University of Padova, Padova

Purpose: We recently found that the Heat Shock Protein of 70kDa (HSP70) is overexpressed in Chronic Lymphocytic Leukemia (CLL) B cells¹. Considering the pro-survival role of HSP70 in cancer², we were aimed at characterizing this protein in the context of the pathogenetic mechanisms leading to CLL.

Methods: HSP70 expression and localization were evaluated by Western blotting and confocal microscopy analyses in B cells from 110 CLL and 26 healthy subjects. HSP70 levels were correlated to clinical parameters and were also analyzed in CLL B cells obtained from patients undergoing treatment with Ibrutinib. The effects of HSP70 and Heat Shock Factor 1 (HSF1) inhibition (by Zafirlukast and Fisetin) were evaluated by Annexin V/Propidium Iodide flow cytometry test.

Results: We found that HSP70 is overexpressed in CLL patients, correlated to poor prognosis and abnormally localized in the nucleus of leukemic vs normal B cells. HSP70 levels decreased in patients responsive to in vivo Ibrutinib treatment. HSP70 and HSF1 inhibition was proved to be effective in inducing a dose-dependent in vitro apoptosis of CLL B cells.

Discussion: HSP70 overexpression and correlation with poor prognosis in CLL patients underline its pivotal role in the regulation of leukemic B cells survival. HSP70 reduction in ex vivo leukemic cells of only patients responding to Ibrutinib-containing regimens would indicate an involvement of HSP70 in drug-resistance phenomena. Although in the past HSP70 has been extensively linked to cancer, little progresses have been made in bringing HSP70 inhibitors to the clinic, because of their potential off-target effects. For this reason, an attractive approach may be the targeting of HSP70 major regulator, HSF1. These data demonstrate the HSP70 involvement in the pathogenesis of CLL and identify HSP70 and HSF1 as putative targets for new therapeutic strategies.

Reference

1. Frezzato F. J Leukoc Biol 2016; 100: 1061-70.

P8.5

HSP70 EXPRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS IS CONTROLLED BY ITS TRANSCRIPTION FACTOR VIA RAS-DEPENDENT PATHWAYS

Federica Frezzato¹, Flavia Raggi¹, Benedetta Accordi², Silvia Bresolin², Filippo Severin¹, Veronica Martini¹, Valentina Trimarco¹, Leonardo Martinello¹, Andrea Visentin¹, Monica Facco¹, Giuseppe Basso², Gianpietro Semenzato¹, Livio Trentin¹

¹Department of Medicine, ²Department of Women's and Children's Health, University of Padova, Padova

Purpose: In Chronic Lymphocytic Leukemia (CLL), the Heat Shock Protein of 70kDa (HSP70) is strictly correlated to its transcription factor Heat Shock Factor 1 (HSF1) [1]. This latter is regulated by a fine balance of activatory/inhibitory phosphorylations mediated by kinases belonging to pathways triggered by RAS (i.e. PI3K/AKT/mTOR and RAF/MEK/ERK) [2]. Our purpose was to gain information and dissect these networks in CLL.

Methods: In a Reverse Phase Protein Array (RPPA) study, previously performed in B cells from 57 CLL patients and 11 healthy subjects, we assessed the activation/expression of key signalling proteins [3]. Herein, we focused on HSP70, AKT-Ser473, mTOR-Ser244, GSK3a/b-Ser21/9, CDK2, CREB-Ser133, MEK1/2-Tyr217-221, ERK1/2-Thr202/Tyr204, NFkB-Ser536, p38MAPK-Thr180/Tyr182, SAPK-JNK-Thr183/Tyr185 and PDK1-Ser241. Cluster and separated analyses have been performed.

Results: The examined proteins behave in a different way between patients expressing high or low levels of HSP70. HSP70-high patients present high Akt-Ser473, an inhibitor of GSK3a/b that, in the inhibited form, prevents HSF1 inhibition. By contrast, HSP70-low patients have high MEK1/2-Ser217/221 and ERK-Thr202/Tyr204, known to negatively regulate HSF1.

Conclusions: These data seem to indicate that, in CLL, HSP70 expression is regulated by the modulation of HSF1 activity through the activation of one or the other way triggered by RAS. In particular, an activation of the PI3K/AKT/mTOR pathway leads, as result, to a higher expression of HSP70 while an activation of the RAF/MEK/ERK signalling rather results in HSP70 down regulation.

The dissection of signalling pathways connected to HSP70-HSF1 axis in CLL will contribute to define the biology and understand the pathogenesis of this disease.

References

1. Frezzato F. Blood 2016; 128: 4368.
2. Downward J. Nat Rev Cancer 2003; 3: 11-22.
3. Frezzato F. J Leukoc Biol 2016; 100: 1061-70.

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P8.7

p66Shc DEFICIENCY PROMOTES CXCR4/CCR7 RECYCLING IN CHRONIC LYMPHOCYTIC LEUKEMIA

Nagaja Capitani¹, Laura Patrucci², Francesca Cattaneo², Noemi Manganaro², Alessandra Gamberucci³, Federica Frezzato⁴, Veronica Martini⁴, Andrea Visentin⁴, Pier Giuseppe Pelicci⁵, Mario Milco D'Elios¹, Livio Trentin⁴, Gianpietro Semenzato⁴, Cosima Baldari²

¹University of Firenze, Firenze

²Department of Life Sciences, ³Department of Molecular and Developmental Medicine, University of Siena, Siena

⁴Department of Medicine, Hematology and Clinical Immunology Branch, University of Padova, Padova

⁵European Institute of Oncology, Milano

Purpose: Neoplastic cell traffic abnormalities are central to the pathogenesis of chronic lymphocytic leukemia (CLL) (1). We previously reported that enhanced CXCR4 and CCR7 recycling contributes to their elevated surface levels on CLL cells (2) and implicated p66Shc, whose expression is defective in these cells (3), in the orchestration of B-cell traffic.

Methods: Here we analyzed CXCR4/CCR7 receptor recycling, cell adhesion, chemotaxis, calcium flux, subcellular chemokine receptors localization and formation of multimolecular complexes using the CLL-derived B-cell line MEC stable transfected for p66Shc expression or B lymphocytes purified from 26 healthy donors or 46 CLL patients, either reconstituted or not for p66Shc expression. RESULTS: p66Shc reconstitution in CLL cells reduces CXCR4/CCR7 recycling, lowering their surface levels and attenuating B-cell chemotaxis, due to their accumulation in Rab5⁺ endosomes as serine-phosphoproteins bound to β -arrestin. This results from the ability of p66Shc to inhibit Ca²⁺ and PP2B-dependent CXCR4/CCR7 dephosphorylation. We also show that the Btk inhibitor ibrutinib reverses the CXCR4/CCR7 recycling abnormalities in CLL cells by increasing p66Shc expression.

Discussion: The evidence presented in this report that p66Shc limits CXCR4 and CCR7 recycling, together with the fact that p66Shc modulates CCR7 and S1PR1 expression (4) as well as CXCR4 signaling (2), underscores the importance of the p66Shc defect in the ability of CLL B cells to home to and accumulate in the protective stromal niche.

Conclusions: These results, identifying p66Shc as a regulator of CXCR4/CCR7 recycling in B cells and provide new clues to mechanisms behind ibrutinib's activity.

References

1. Burger JA et al. Semin Cancer Biol 2014; 24: 71-81.
2. Patrucci L et al. Cancer Res 2015; 75: 4153-63.
3. Capitani N et al. Blood 2010; 115: 3726-36.
4. Capitani N et al. Blood 2012; 120: 4391-9.

P8.8

ECTOPIC EXPRESSION OF ILT3 CONTROLS BCR-DEPENDENT ACTIVATION OF AKT IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

Vanessa Zurli¹, Giuliana Wimmer¹, Veronica Candi², Emanuele Cencini², Alessandro Gozzetti², Francesca Cattaneo¹, Francesca Sanseviero¹, Monica Bocchia², Cosima Tatiana Baldari¹, Anna Kabanova¹

¹Department of Life Sciences, ²Hematology Unit, University of Siena, Siena

Purpose: The high proportion of long term non-progressors among CLL patients suggests the existence of a regulatory network which restrains the proliferation of tumor B cells. The identification of determinants composing such network is hence fundamental for our understanding of CLL pathogenesis. We have previously established that CLL cells are deficient in the signaling adaptor p66Shc. Here we undertook to identify unique phenotypic traits caused by this defect and analyzed its functional significance.

Methods: We used transcriptomics, protein expression profiling and signaling studies to characterize ILT3, an immunoglobulin-like transcript 3, as a signature molecule of p66Shc deficiency in CLL cells.

Results: We found that lack of p66Shc in CLL cells modulated their transcriptome and promoted an upregulation of the surface receptor ILT3, which is normally found on myeloid cells. The ectopic expression of ILT3 in CLL was a distinctive feature of B cells and hematopoietic stem cells, thus identifying ILT3 as a marker of circulating CLL cells and their progenitors in the bone marrow. ILT3 expression in CLL was driven by Deltex1, a regulatory suppressor of antigen receptor signaling in lymphocytes. Consistently, we found that ILT3 had a regulatory function in CLL cells since its triggering inhibited the activation of AKT upon BCR stimulation. This effect was achieved through the dynamic coalescence of ILT3, BCRs and inositol phosphatase SHIP-1 into inhibitory clusters at the cell surface.

Discussion: Activation of the AKT provides a powerful mitogenic signal to B cells, therefore its tight regulation by ILT3 could provide a means to control B cell activation in CLL.

Conclusions: Collectively, our findings define the mechanisms which promote the ectopic expression of ILT3 in CLL cells and suggest that it may functionally contribute to a regulatory network controlling tumor progression by suppressing the AKT pathway.

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P8.9

BORTEZOMIB REDUCES INTERLEUKIN 6 PRODUCTION AND IMPAIRS CELLS SURVIVAL IN LARGE GRANULAR LYMPHOCYTES LEUKEMIA

Giulia Calabretto¹, Antonella Teramo¹, Gregorio Barilà¹, Chiara Ercolin¹, Matteo Leoncin¹, Cristina Vicenzetto¹, Anna Cabrelle¹, Monica Facco¹, Gianpietro Semenzato¹, Renato Zambello¹

¹Departement of Medicine, University of Padova, Padova

Purpose: Large Granular Lymphocyte Leukemia is a lymphoproliferative disorder characterized by the clonal expansion of Large Granular Lymphocytes (LGLs). Teramo et al. (2013) demonstrated an increased level of Interleukin(IL)-6 in LGL Leukemia plasma patients. IL-6 is a downstream target of NF- κ B transcription factor and plays a role in LGLs proliferation and survival through JAK/STAT pathway activation [1]. The proteasome inhibitor Bortezomib leads to NF- κ B suppression, impairing cellular release of pro-inflammatory cytokines [2]. The aim of the study is to investigate Bz in-vitro effects on IL-6 production and on LGLs survival.

Methods: 20 LGL Leukemia samples were treated with Bz (5.2 nM) for 24-48 hours. Cell survival was evaluated by Annexin V staining. Transcriptional and protein levels were evaluated by Flow Cytometry (FC), Real time PCR and Western Blot (WB) assays.

Results and Discussion: Our results showed a complete depletion of monocytes after 24hours of Bz treatment, followed by a significant time-dependent increase of LGLs apoptosis, as compared to control ($p < 0.01$). To evaluate IL-6 production, we measured IL-6 protein levels by FC in monocytes after 18h of Bz treatment and we observed a 60 % reduction as compared to control, demonstrating Bz anti-inflammatory properties. To investigate Bz effects on IL-6-induced JAK/STAT pathway, we performed WB analysis to evaluate STAT3 activation, showing that pSTAT3 Tyr705 levels were strongly decreased in Bz-treated conditions. Therefore, we investigated the effects of STAT3 deactivation on the anti-apoptotic genes MCL-1 and BCL-2, two main STAT3 downstream targets. Our data demonstrated a reduction in transcriptional and protein levels of both these genes, of 54% for MCL-1 and 61% for BCL-2.

Conclusion: Bz might represent a new therapeutic option for LGL Leukemia therapy.

References

1. Teramo A et al. Blood 2013; 121: 3843-54.
2. Mohty M et al. Biol Blood Marrow Transplant 2013; 19: 1416-20.

P8.10

LACK OF p66Shc IN THE CLL MOUSE MODEL E μ -TCL1 EXACERBATES NODAL AND EXTRANODAL INFILTRATION OF LEUKEMIC CELLS WHICH CAN UNDERGO RICHTER'S TRANSFORMATION

Laura Patrussi¹, Nagaja Capitani², Cristina Olivieri¹, Francesca Cattaneo¹, Noemi Manganaro¹, Massimo Granai³, Stefania Gobessi⁴, Dimitar Efremov⁴, Lorenzo Leoncini³, Cosima T. Baldari¹

¹Department of Life Sciences, University of Siena, Siena

²Department of Clinical and Experimental Medicine, University of Firenze, Firenze

³Department of Human Biotechnologies, University of Siena, Siena

⁴Molecular Hematology group, ICGEB, Trieste

Purpose: Chronic lymphocytic leukemia (CLL) cells have a defective expression of the p66Shc adaptor which correlates with poor prognosis and disease severity¹. The impairment in p66Shc expression alters the balance between homing and egress receptors^{2,3}, favouring the accumulation of leukemic cells in the pro-survival stromal microenvironment. Here we analyzed the impact of p66Shc gene disruption on disease severity and progression.

Methods: We crossed E μ -TCL1 and p66Shc^{-/-} to generate the new E μ -TCL1/p66Shc^{-/-} mouse strain. We analyzed by flow cytometry blood samples from 140 mice of both strains to assess the percent of CD5⁺/IgM⁺/CD19⁺ tumoral cells. We stained tissues from both nodal and extranodal sites with hematoxylin/eosin to analyze tumoral infiltration and performed qRT-PCR of chemokine receptors in tumoral cells.

Results: We found that E μ -TCL1/p66Shc^{-/-} mice develop an aggressive disease that has an earlier onset, a higher incidence and leads to an earlier death compared to E μ -TCL1 mice. A significant proportion of E μ -TCL1/p66Shc^{-/-} mice displays substantial accumulation of leukemic cell not only at nodal but also at extranodal sites, which subverts organ architecture and in several instances develops into aggressive lymphomas, similar to Richter's transformation of CLL. We moreover found an upregulation in the expression of chemokine receptors whose ligands are known to be enriched at these sites.

Discussion: E μ -TCL1/p66Shc^{-/-} develop a disease with an earlier onset and a worst prognosis. This is relatable to the increased expression of chemokine receptors which accounts for the infiltration of p66Shc^{-/-} leukemic cells in nodal and extranodal sites.

Conclusion: Our results highlight p66Shc as a key regulator of CLL-like disease progression and severity in the TCL1 mouse model and identify E μ -TCL1/p66Shc^{-/-} mice as a new valuable model of aggressive CLL.

References

1. Capitani et al. Blood 2010.
2. Capitani et al. Blood 2012.
3. Patrussi et al. Cancer Res 2015.

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P9 - CYTOKINES AND CHEMOKINES

P9.1 CHEMOKINE RECEPTORS EXPRESSION ON CIRCULATING MONOCYTES AND NEUTROPHILS IN HIGH GRADE GLIOMA PATIENTS

Matteo Massara¹, Enrico Lugli², Marco Conti Nibali²,
Marco Rossi², Lorenzo Bello², Matteo Simonelli²,
Massimo Locati¹, Raffaella Bonecchi³

¹Humanitas Clinical and Research Center, Rozzano, Italy;
Department of Medical Biotechnologies and Translational
Medicine, Università degli Studi di Milano, Rozzano

²Humanitas Clinical and Research Center, Rozzano

³Humanitas Clinical and Research Center, Rozzano; Humanitas
University, Rozzano

Purpose: High grade gliomas (HGGs) are the most frequent brain tumors and include the more aggressive form glioblastoma multiforme (GBM) with short survival time after diagnosis. One of the characteristics of HGGs is the loss of blood-brain barrier and the vascularization of the tumoral tissue. Leukocytes infiltrate the tumor and are key modulators of tumor growth and responsiveness to therapy. The objective of the study is to correlate circulating myeloid cells phenotypes to HGG aggressiveness.

Methods: Blood was collected before the surgery and compared to age-matched healthy control. Circulating myeloid cells were analyzed by multiparametric flow cytometry technique.

Results: HGG patients had an increase rate of classical CD14+ monocytes compared to the non-classical CD16+ subset with a dysregulated expression of inflammatory chemokine receptors. We also found three different neutrophil subpopulations with differential expression of activation state markers and chemokine receptors in HGG patients compared to healthy controls.

Discussion: Multiparametric analysis of circulating myeloid populations indicate that in HGG patients both monocyte and neutrophil subpopulations were present in unbalance rate with dysregulated expression of activation markers and chemokine receptors.

Conclusions: the presence of differentially activated myeloid cells in the blood of HGG patients could be used as a prognostic indicator of HGG aggressiveness.

References

1. Bonavita O et al. Cytokine Growth Factor Rev 2016; 30: 81-6.
2. Pessina F et al. Value of Surgical resection in patients with newly diagnosed grade III glioma treated in a multimodal approach: surgery, chemotherapy and radiotherapy. Ann Surg Oncol 2016; 23: 3040-6.
3. Scorsetti M et al. Multimodality therapy approaches, local and systemic treatment, compared with chemotherapy alone in recurrent glioblastoma. BMC Cancer 2015; 30: 15:486.

P9.2

CYTOKINES-LYMPHOID PROGENITORS INTERPLAY IN TREATED ACUTE HIV INFECTION

Veronica Bordonì¹, Domenico Viola¹, Alessandra Sacchi¹, Rita Casetti¹, Eleonora Cimini¹, Nicola Tumino¹,
Federico Martini¹, Carmela Pinnetti², Andrea Antinori², Adriana Ammassari², Chiara Agrati²

¹Cellular Immunology Lab, ²Clinical Department, INMI
L.Spallanzani, Roma - Italy

Purpose: Lymphoid hematopoietic progenitors cells (L-HPC) play a pivotal role in determining immune reconstitution capability. Very recently we demonstrated a relationship between pro-inflammatory cytokines and L-HPC frequency in both naïve primary and chronic HIV infection. Aim was to evaluate the impact of antiretroviral treatment (cART) on the balance between L-HPC and plasmatic IL-7, IL-18 as well as Stem Cell Factor (SCF) during primary HIV infection (PHI).

Methods: Plasma/PBMC samples from 44 pts included in the SIREA study were used. Quantification of IL-7, IL-18 and SCF was evaluated by Luminex before and after 6 months of cART. Circulating L-HPC frequency, defined as CD45RA+CD10+CD117- within CD34+Lin- population, was performed by FACS. Correlation analysis was performed by Spearman test.

Results: After 6 months of cART, median HIV-RNA was <40 copies/mL and median CD4 rose to 820/mm³. When compared to baseline, at 6 months a significant increase of L-HPC frequency and CD4 cell count was observed, while levels of pro-inflammatory IL-18 and homeostatic IL-7 cytokines decreased significantly. Conversely, SCF level was higher after 6 months of cART in respect to baseline. Interestingly, L-HPC frequency directly correlated to IL-18 levels at baseline and to SCF levels after 6 months of therapy. Differently to data reported in naïve chronic HIV infection, no correlation was found between L-HPC frequency and IL-7 levels in PHI before or after 6 months of cART.

Discussion and Conclusions: In PHI, cART modifies the interplay between cytokines and L-HPC homeostasis. Indeed, L-HPC frequency is sustained by IL-18 in the naïve patient, and by SCF after start of cART. These insights may open new perspectives for the evaluation of these signalling pathways for future potential therapeutic approaches at different stages of HIV infection.

References

1. Bordonì V et al. Clin Infect Dis 2016.
2. Sauce D et al. Blood 2011.
3. Levy Y. J Clin Invest 2009.

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P9.3

A PARACRINE CIRCUIT INVOLVING C-KIT AND STEM CELL FACTOR PROMOTES CANCER STEM CELL SURVIVAL IN OVARIAN CANCER

Anna Pastò¹, Giorgia Pilotto¹, Elena Laura Mazzoldi², Maria Ornella Nicoletto¹, Alberto Amadori²

¹Istituto Oncologico Veneto-IOV, Padova

²University of Padua, Department of Surgery, Oncology and Gastroenterology, Padova

Stem cell factor (SCF) is a protein, physiologically present in either membrane or soluble form [1], involved in cell proliferation and differentiation. SCF binds its receptor CD117 (c-kit), marker of cancer stem cells (CSC) in several tumor tissues including ovary [2,3]. Little is known regarding SCF in epithelial ovarian cancer (EOC). Thus, we decided to investigate SCF production and its role in tumor cells from ascitic effusions of EOC patients.

SCF was evaluated by ELISA, qRT-PCR and flow cytometry in sorted CD117⁺ (CSC), CD117⁻ (non-CSC), tumor-associated macrophages (TAM) or fibroblasts (TAF).

ELISA analysis revealed a high amount of soluble SCF in the ascitic fluids, which was produced only by TAM and TAF. Flow cytometry analysis of membrane SCF demonstrated SCF^{pos} cells within both CSC, non-CSC, TAM and TAF. These results were confirmed by qRT-PCR analysis: only TAM and TAF expressed both the membrane and the complete SCF protein. Co-culture of CSC with TAM, or their supernatant, activated c-kit as demonstrated by AKT phosphorylation. We next tested the effects of recombinant SCF (hrSCF) on stemness features of EOC cells. hrSCF treatment significantly increased the ratio of spheroid-forming cells, the percentage of CD117⁺ cells, the mRNA levels of the stemness-associated genes Nanog, Sox2 and Oct4, and the CSC tumorigenic potential compared to untreated cells. All these effects were prevented by Imatinib treatment.

We demonstrated that SCF is expressed by EOC cells only in a membrane form, whereas it is also secreted by TAM and TAF. SCF promotes all the stemness features, impaired by Imatinib treatment. In conclusion, our data suggest that within EOC microenvironment both homotypic and heterotypic interactions between stromal, immune cells and CSC may occur, based on a juxtacrine/paracrine circuit that involves soluble and membrane-bound SCF and its receptor.

References

1. Lennartsson J et al. Stem cell factor receptor/c-kit: from basic science to clinical implications. *Physiol Rev* 2012; 92: 1619-49.
2. Pastò A et al. Cancer stem cells from epithelial ovarian cancer patients privilege oxidative phosphorylation and resist glucose deprivation. *Oncotarget* 2014; 30: 4305-19.
3. Zhang S et al. Identification and characterization of ovarian cancer-initiating cells from primary human tumors. *Cancer Res* 2008; 68: 4311-20.

P9.4

COMMON EFFECTS OF IFN γ AND IL27 ON THE HLA CLASS I ANTIGEN PRESENTATION MACHINERY IN HUMAN CANCER CELLS

Grazia Carbotti

AOU San Martino-IST, Genova

Purpose: IL27, a member of the IL12-family, has shown anti-tumor activity in pre-clinical models in relationship to immune-enhancing effects and ability to inhibit tumor proliferation and angiogenesis. However, IL27, similar to IFN γ , induces the expression of IL18BP [1], IDO and PDL1 [2] immune regulatory molecules in human cancer cells. Here, we further explored the functional overlaps between IL27 and IFN γ through a proteomic approach.

Methods: Human ovarian cancer cell lines stimulated with IL27 or IFN γ or left untreated were lysed and their protein expression was analyzed by the mass spectrometer LTQ-Orbitrap Velos Pro. The raw data were processed with MaxQuant software and bioinformatics with Perseus [3].

Results: Among 990 proteins modulated by cytokine treatment in SKOV3 cells, 814 showed a concordant modulation by both cytokines, while only 176 showed cytokine-specific changes. The most up-regulated proteins were common and included the IFN mediators STAT1, IFIT1 and 3, GBP1, 2 and 5, and the enzymes IDO, GAMT, WARS, OAS1 and -3. Importantly, several IL27 up-regulated proteins belong to the proteasome or the HLA class I APM. Functional analysis of IL27-regulated networks highlighted pathways of IFN signaling, antigen presentation, protection from NK cell cytotoxicity, proteasome, amino acid catabolism and regulation of viral protein levels. Importantly, IL27 induced surface HLA class I molecule expression in different human cancer cells, including tumor cells showing very low expression such as neuroblastoma.

Conclusions: Altogether, these data point out to a broad set of activities shared by IL27 and IFN γ , which are dependent on the common activation of the STAT1 pathway. These data add further explanation to the anti-tumor activity of IL27 and also to its dual role in immune regulation.

References

1. *Clin Cancer Res* 2013; 19: 4611-20.
2. *Oncotarget* 2015; 6: 43267-80.
3. *Oncotarget* 2016; 7: 72518-36.

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P9.5 MODIFICATIONS OF IL-17 TYPE CYTOKINES IN PATIENTS WITH HEREDITARY ANGIOEDEMA AS EXPRESSION OF A SYSTEMIC ACTIVATION PROCESS

Francesco Arcoleo¹, Mariangela Lo Pizzo¹, Enrico Cillari²

¹Cervello Hospital-AOR Villa Sofia-Cervello, Palermo

²European Medical Institute, Palermo

Introduction: Hereditary angioedema (HAE) is a rare autosomal dominant disorder characterized by a deficiency of C1 esterase inhibitor (C1INH) which causes episodic swellings of subcutaneous tissues, bowel walls and upper airways that are disabling and potentially life-threatening.

Materials and Methods: We evaluated n = 17 patients with confirmed HAE diagnosis in basal and crisis state and n=19 healthy subjects. The samples were tested for a panel of IL-17 type cytokines (interleukin (IL)-1b, IL-6, IL-10, Granulocyte Macrophage Colony Stimulating Factor (GM-CSF), IL-17, IL-21, IL-22, IL-23, Transforming Growth Factor-beta - TGF- β subtypes), using Bio-plex kit (BioRad, Milano, Italy). Data analysis was performed linear mixed models with censored response to account for limits of detection of some cytokines levels.

Results: The results indicate the variations of cytokine levels in HAE subjects comparing the condition during the crisis with the basal value. Out of the 11 analysed cytokines, we found out significant (i.e p-value < 0.05) differences between crisis and basal state for IL-1b, IL-6, IL-10, IL-17, IL-21, GM-CSF, TGF β 1 and TGF β 2 cytokines (fig.1). Altogether, the modifications of all these cytokines during the acute attacks compared with the basal values, indicate that type 17 signature cytokines (IL-17, GM-CSF, IL-21, IL-1 β , IL-6) and TGF β 1 and TGF β 2 together with IL-10 are increased, whereas IL-23 is unmodified and TGF β 3 is significantly reduced. When comparing healthy and HAE subjects at basal state, we found a significant difference for IL-17, GM-CSF, IL-21, TGF β 1, TGF β 2 cytokines.

Discussion: In this study comparing angioedema patients before and after the acute crisis with matched controls, we observed several significant modifications of lymphokine network. The most prominent up-regulated cytokines were GM-CSF, IL-17, IL-21, TGF β 1 and TGF β 2 before the onset of crisis, whereas significant elevations of IL-6, IL-17, IL-10, GM-CSF, IL-21, TGF β 1 and TGF β 2 were detected during the onset of the crisis. These results confirm and extend previous our findings indicating that in HAE there is operating a systemic activation process which involves T helper 17 (Th17) cytokines and TGF β isoforms that results in localized angioedema attack characterized by local elevated bradykinin (BK) levels. It has become widely accepted that during HAE attacks permeability is increased by binding of bradykinin to BKR-2 and possibly BKR-1 activating signaling cascades resulting in production of vasodilating mediators.

P9.6 SYNERGISTIC PRODUCTION OF TNF α AND IFN α BY HUMAN pDCs INCUBATED WITH IFN λ 3 AND IL-3

Giulia Finotti¹, Nicola Tamassia¹, Marco Antonio Cassatella¹

¹Department of Medicine, University of Verona, Verona

Purpose: The interplay between plasmacytoid dendritic cells (pDCs) and members of the IFN λ family is becoming increasingly relevant. Recently, we reported (1) that human pDCs incubated with IFN λ 3 prolong their survival, alter the expression of maturation markers and produce variable amounts of IFN α , CXCL10 and TNF α . We also observed that both IFN λ 3 and IL-3 upregulate the expression of CD123/IL-3Ra and IFNIR1, suggesting that they may reciprocally influence pDC responsiveness to each other.

Methods: pDCs were isolated from PBMCs of buffy coats of healthy donors and cultured in the presence or absence of IFN λ 3 and/or IL-3, with or without etanercept, adalimumab or aIFN α R Abs. Supernatants were harvested for cytokine measurements, while the corresponding cell pellets were used for flow cytometry analysis or lysed for RNA extraction.

Results: We now report (2) that: combination of IFN λ 3 and IL-3 synergistically induces the expression of Interferon-Stimulated Gene (ISG) mRNA and the production of TNF α and IFN α ; endogenous IFN α autocritically promotes the expression of ISG mRNAs in IL-3-, but not in IFN λ 3 plus IL-3-, treated pDCs; production of IFN α by IFN λ 3 plus IL-3-treated pDCs is mostly dependent on endogenously produced TNF α .

Discussion: This study demonstrates that IFN λ 3 and IL-3 reciprocally influence their capacity to activate various functions of human pDCs, such as the production of IFN α and TNF α , as well as the expression of ISG mRNA. Using neutralizing antibodies, we also demonstrate that the production of IFN α by IFN λ 3 plus IL-3-treated pDCs is mostly dependent on endogenously produced TNF α , consistent with a crucial role of pDC-derived TNF α in autocritically amplifying the production of cytokines, as reported for CXCL10 (1).

Conclusions: Data suggest that IFN λ 3 and IL-3 collaborate to promote discrete functional responses of human pDCs at maximal levels. Data also point for novel role of IL-3 in enhancing IFN λ -activated pDC responses.

References

1. Finotti G et al. J Leukoc Biol 2016; 99: 107-19.
2. Finotti G et al. Cytokine 2016; 86: 124-31.

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P9.7

ANGIOGENIC AND FIBROTIC MEDIATORS IN IGG4-RELATED DISEASE

Riccardo Capecechi¹, Federico Pratesi¹, Antonio Tavoni¹, Paola Migliorini¹, Ilaria Puxeddu¹

¹Department of Clinical and Experimental Medicine, Pisa

Background: IgG4-related disease (IgG4-RD) is a fibroinflammatory condition that can affect almost any organ, characterized by lymphoplasmacytoid infiltrate, obliterative phlebitis and storiform fibrosis often associated with eosinophilia and increased levels of IgG4. Cytotoxic CD4 T cells producing IL-1b, TGFb1 and IFN-g are detectable in peripheral blood of patients and high IL-18 expression has been found in affected organs.

Objectives: To evaluate the role of IL-1 family cytokines in IgG4-RD, analyzing cytokine and receptors in sera.

Methods: Nine patients fulfilling the proposed criteria for the diagnosis of IgG4-RD were recruited. Cytokines of the IL-1 family (IL-1a, IL-1b, IL-33, IL-18), soluble receptors (sIL-1R1, sIL-1R2, sIL-1R3, sIL-1R4) and antagonists (IL-1Ra, IL-18 binding protein) were measured in sera by multiarray ELISA assay. Free IL-18 was calculated using the law of mass action.

Results: IL-18 ($p = 0.007$) and free IL-18 ($p < 0.0001$), sIL-1R1 ($p = 0.0005$), sIL-1R2 ($p = 0.0013$), sIL-1R4 ($p = 0.0006$) were significantly increased in IgG4-RD sera compared with healthy controls.

Conclusions: In IgG4-RD patients, at variance with other autoimmune or autoinflammatory conditions, the increase in IL-18 levels is not counterbalanced by IL-18BP, leading to high levels of free IL-18. The free cytokine may affect T cell subset balance and induce IFN-g production. The parallel increase of sIL-1R1 and sIL-1R2 suggests an efficient dampening of proinflammatory IL-1b signaling, while high levels of sIL-1R4 may be associated with vascular remodeling and fibrosis, as observed in animal models of obesity and in human cardiovascular disorders.

P10 - SEVERE ASTHMA

P10.1

ANALYSIS OF OMALIZUMAB INFLUENCE ON MAST CELL-BOUND IGE

Flavia Maria Frisenda¹, Anna Simona Rucco¹, Irene Fiorino¹, Georgios Kourtis¹, Filomena Loconte¹, Carlo Pasculli¹, Maria Luisa Taurino², Luciana D'Elia², Marcello Albanesi¹, Danilo Di Bona¹, Maria Filomena Caiaffa², Luigi Macchia¹

¹Cattedra e Scuola di Specializzazione di Allergologia ed Immunologia Clinica, Università di Bari - Aldo Moro, Bari

²Cattedra e Scuola di Specializzazione di Allergologia ed Immunologia Clinica, Università di Foggia, Foggia

Purpose: The 2014 GINA Guidelines recommend the use of omalizumab (an anti-IgE Mab) in step 5 treatment in patients with severe asthma, in addition to inhaled corticosteroid and long-acting beta agonist at high doses. The objective of this study was to analyze the changes of mast cell-bound specific IgE (assessed in terms of quantitative skin prick test (qSPTs) wheal size area). The levels of serum total IgE measured before and during administration of omalizumab were also investigated.

Methods: In a population of 91 patients from two Italian Hospitals, treated with omalizumab up to 9 years, we performed qSPTs for inhalant allergens (dust mite, cat, dog, horse, Aspergillus fumigatus, pollens from olive, grasses, Parietaria, cypress, ash tree, Artemisia). We measured total IgE levels by PRIST.

Results: Comparing the mean values of wheal size areas, we noticed a substantial reduction when results obtained before starting omalizumab were compared with that of the last available measurement (from 1 to 9 years), which was statistically significant for dust mite (*Dermatophagoides pteronyssinus* ($p = 0.02$) and *D. farinae* ($p = 0.004$)) and pollens (*parietaria* - $p = 0.004$, *cypress* - $p = 0.04$, *artemisia* - $p = 0.02$). A decline in total IgE levels over time was also documented.

Discussion: Considering that IgE bound to the high affinity receptors (FcεRI) on the surfaces of mast-cells are in balance with free IgE, the removal of circulating IgE by omalizumab also influence their presence on mast-cells, causing, as a consequence, a reduced response upon allergen stimulation, which can be observed by measuring qSPTs wheal areas.

Conclusions: qSPTs can be considered a reliable way for evaluating the direct influence of omalizumab on IgE levels.

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P10.2

THE EFFECTS OF MONTELUKAST AND ZILEUTON ON BRONCHIAL HYPERRESPONSIVENESS, AIRWAY INFLAMMATION AND AIRWAY SMOOTH MUSCLE REMODELING

Marina Syrczova¹

¹Department of general and private morphology, FSRU "Institute of Experimental Medicine", St-Petersburg - Russian Federation

Purpose: This research aimed evaluates the effects of montelukast and zileuton on bronchial hyperresponsiveness, airway inflammation and airway smooth muscle remodeling.

Methods: This research includes six groups of animals (n = 50, m = 210-230 gr). The 1st group included the control animals, the 2nd group included the intact animals (with insufflation lactose), 3rd group -insufflation montelukast (10 mg / kg), the 4th group-zileuton (10 mg / kg), the 5th group - the standard therapy Salmeterolum + Fluticasonum (10mg / kg) and the 6th group, the modeling of bronchial asthma. The method of administration was dry insufflation.

Results: In the group with asthma, the number of inflammatory cells in BALF eosinophils was 2.16 ± 0.03 ; mucus hypersecretion: increased in the number of goblet cells, increased mucus secretion: $32.2 \pm 8.3 \text{ mm}^2$; changes in the blood picture: leukocytosis (3.09 ± 0.03). Collagen around airways: $41.2 \pm 6.7 \text{ mm}^2$. ($p < 0.01$). The results in the montelukast group were: 1.76 ± 0.23 ; $5 \pm 2 \text{ mm}^2$; 2.31 ± 1.20 ; $12.7 \pm 3.4 \text{ mm}^2$ respectively. In the group of zileuton: 1.21 ± 0.18 ; $3 \pm 1 \text{ mm}^2$; 2.00 ± 0.68 ; $8.7 \pm 2.1 \text{ mm}^2$ respectively.

Discussion: Montelukast and zileuton are leukotriene antagonist of growing interest as an alternative therapy for asthma across different age groups due to its bronchoprotective, anti-inflammatory and anti-allergic properties [1].

Conclusion: Thus, in addition to the basic pharmacological effect these drugs act indirectly on the main elements of the allergic response in experimental asthma that make this group a promising drug for the treatment of patients with severe asthma.

Reference

1. Janeva EJ, Goseva Z, Gjorchev A, et al. The Effect of Combined Therapy ICS/LABA and ICS/LABA plus Montelukast in Patients with Uncontrolled Severe Persistent Asthma Based on the Serum IL-13 and FEV1. Open access Macedonian Journal of Medical Sciences 2015; 3: 268-72.

P11 - T CELL ITEMS

P11.1

ISA-2011B, A PHOSPHATIDYLINOSITOL 4-PHOSPHATE 5-KINASE A INHIBITOR, IMPAIRS CD28-DEPENDENT COSTIMULATORY AND PRO-INFLAMMATORY SIGNALS IN HUMAN T LYMPHOCYTES

Martina Kunkl¹, Nicola Porciello¹, Marta Mastrogianni¹, Cristina Capuano², Federica Lucantoni², Chiara Moretti², Jenny L. Persson³, Ricciarda Galandrini², Raffaella Buzzetti², Loretta Tuosto¹

¹Istituto Pasteur-Fondazione Cenci Bolognetti, Department of Biology and Biotechnology Charles Darwin, Sapienza University, Roma

²Department of Experimental Medicine, Sapienza University, Roma

³Division of Experimental Cancer Research, Department of Laboratory Medicine, Clinical Research Center, Lund University, Malmö - Sweden

Purpose: Phosphatidylinositol 4,5-bisphosphate (PIP₂) is a cell membrane phosphoinositide that, in T lymphocytes, controls the activity of several proteins regulating cytoskeleton reorganization, cytokine gene expression, T cell survival, proliferation and differentiation. The main biosynthetic pathway of PIP₂ involves phosphorylation of phosphatidylinositol 4-monophosphate (PI4P) by phosphatidylinositol 4-phosphate 5-kinases (PIP5Ks). In human T lymphocytes, we have recently demonstrated that CD28 is the crucial costimulatory receptor that regulates PIP₂ turnover by recruiting and activating PIP5K α . We also found that PIP5K α is the main regulator of CD28 costimulatory and autonomous functions (1,2). Since it has been recently discovered a specific inhibitor of PIP5K α , ISA-2011B (3), herein we characterized its inhibitory effects on T lymphocyte functions in HLA-transgenic (HD) and Type 1 Diabetes (T1D) patients.

Methods: Kinase assay was performed to characterize the effect of ISA-2011B on the lipid kinase activity of PIP5K α in both Jurkat and primary T cells; luciferase assays were used to assess NF- κ B and NF-AT activation; RT-qPCR was performed to evaluate cytokine production, upon CD28 stimulation, in HD and T1D patients.

Results and Discussion: Inhibition of PIP5K α lipid kinase activity by ISA-2011B significantly impaired CD28 costimulatory signals integrating those delivered by TCR as well as CD28 autonomous signalling regulating the expression of pro-inflammatory cytokines and chemokines in human T lymphocytes. Moreover, we found that ISA-2011B also impaired CD28-mediated up-regulation of inflammatory cytokines in Type 1 Diabetes (T1D) patients.

Conclusions: Our data on the strong inhibitory effect exerted by ISA-2011B on T lymphocytes, suggest that targeting the CD28/PIP5K α signalling pathway may provide an attractive therapeutic approach for the resolution of inflammatory processes in autoimmune diseases.

References

1. Muscolini M, Camperio C, Capuano C, Caristi S, Piccolella E, Galandrini R, Tuosto L. Phosphatidylinositol 4-phosphate 5-kinase α activation critically contributes to CD28-dependent signaling responses. J Immunol 2013; 190: 5279-86. doi:10.4049/jimmunol.1203157
2. Muscolini M, Camperio C, Porciello N, Caristi S, Capuano



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- C, Viola A, Galandrini R, Tuosto L. Phosphatidylinositol 4-Phosphate 5-Kinase alpha and Vav1 Mutual Cooperation in CD28-Mediated Actin Remodeling and Signaling Functions. *J Immunol* 2015; 194: 1323-33. doi:10.4049/jimmunol.1401643
3. Semenas J, Hedblom A, Miftakhova RR, Sarwar M, Larsson R, Shcherbina L, Johansson ME, Harkonen P, Sterner O, Persson JL. The role of PI3K/AKT-related PIP5K1alpha and the discovery of its selective inhibitor for treatment of advanced prostate cancer. *Proc Natl Acad Sci U S A* 2014; 111 (35): E3689-98. doi:10.1073/pnas.1405801111

P11.2

HIV-SPECIFIC CD8 T CELLS PRODUCING MACROPHAGE INFLAMMATORY PROTEIN-1 β ARE ASSOCIATED TO WORSE IMMUNE RECONSTITUTION DURING CHRONIC INFECTION

Rita Casetti¹, Carmela Pinnetti², Alessandra Sacchi¹, Gabriele De Simone¹, Veronica Bordonì¹, Eleonora Cimini¹, Nicola Tumino¹, Francesca Besi¹, Domenico Viola¹, Federica Turchi¹, Valentina Mazzotta², Andrea Antinori², Federico Martini¹, Adriana Ammassari², Chiara Agrati¹

¹Laboratory of Cellular Immunology and Pharmacology,

²Clinical Department, National Institute for Infectious Diseases "Lazzaro Spallanzani", IRCCS, Roma

Purpose: Immunological non response represents the Achilles heel in the combination antiretroviral treatment (cART) effectiveness, and increases risk of clinical events and death. CD8 T cells play a crucial role in controlling HIV replication and polyfunctional HIV-specific CD8 T cells have been associated with non-progressive HIV infection. However, the possible role of polyfunctional CD8 T cells in predicting post-treatment immune reconstitution has not yet been explored. Aim of this study was to identify functional markers predictive of immunological response to cART in chronic HIV-infected patients.

Methods: A cohort of chronic HIV-infected individuals naive to cART were enrolled in the ALPHA study. CD4/CD8 T cell subsets, their differentiation/activation, as well as susceptibility to apoptosis were analyzed before and after 12 months of cART. Moreover, CD8 T cells polyfunctional response after HIV antigenic stimulation was also assessed.

Results: Results showed a significant correlation between worse CD4 T cell restoration and low frequency of Naïve CD4 T cells, high frequency of Effector Memory CD4 T cells, and high susceptibility to apoptosis of CD4 T cells all before cART. Moreover, CD8 functional subsets expressing total macrophage inflammatory protein-1 β (MIP-1b) or in combination with CD107a and interferon gamma (IFN γ) were negatively associated to immune reconstitution.

Discussion: Our study shows that a more differentiated phenotype of CD4 T cells and MIP-1b-producing CD8 T cells could represent valuable predictors of worse immune reconstitution.

Conclusions: In conclusion, these parameters may be used as tools for identifying patients at risk of immunological failure during cART and eventually represent the basis for innovative therapeutic strategies.

References

1. Betts MR et al. *Blood* 2006; 107: 4781-89.
2. Nemes E et al. *AIDS* 2010; 24: 947-57.
3. Rehr M et al. *J Virol* 2008; 82: 3391-404.

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P11.3

GENOME WIDE ANALYSIS REVEALS NEW FACTORS CONTRIBUTING TO HUMAN TH17 CELL DIFFERENTIATION PROGRAM

Alessia Capone¹, Manuela Bianco¹, Chiara Naro², Marco De Bardi¹, Luca Battistini¹, Elisabetta Volpe¹, Claudio Sette²

¹Neuroimmunology Unit, ²Neuroembryology Unit, Santa Lucia Foundation, Roma

Purpose: T helper (Th)17 cells are CD4 T cells characterized by production of interleukin (IL)17A and F (1,2). IL-1b, IL-6, IL-23 and TGF- β are all required for differentiation of human Th17 cells (3). However, the differentiation program of these cells is not fully understood. Our study aims at elucidating new mechanisms contributing to human Th17 cell generation and potentially involved in the acquisition of their pathogenic features.

Methods: We generated human Th17 cells from naïve CD4 T cells in optimal condition (IL-1b, IL-6, IL-23 and TGF- β) and Th cells generated in a suboptimal Th17 condition, containing all cytokines except IL-1b. We performed a next-generation RNA sequencing on these cells at 48 hours and 5 days of differentiation. We validated genes by quantitative RT-PCR, and we selected factors whose function has been studied by silencing its expression with siRNA approach.

Results: Our analysis revealed that most genes discriminating Th17 differentiated from undifferentiated cells are induced at 5 days. By comparing genes modulated at 48 hours and 5 days, we identified genes that are involved in Th17 differentiation process at early, late and both time points. Among these last genes, we selected factors whose expression has never been described in Th17 cells, and we demonstrated for the first time their role in the acquisition of specific features of Th17 cells.

Discussion: This study revealed new mechanisms involved in the generation of human Th17 cells. These findings have important implications in facilitating the understanding of Th17 cells and their pathogenic role in autoimmune diseases such as multiple sclerosis (4).

Conclusions: In conclusion, we identified new factors that regulate human Th17 features and that could be targeted for the treatment of Th17-mediated autoimmune diseases.

References

1. Park H et al. Nat Immunol 2005.
1. Harrington LE et al. Nat Immunol 2005.
1. Volpe E. Nat Immunol. 2008.
1. Volpe E et al. Mediators Inflamm 2015.

P11.4

A PHENOTYPICAL AND FUNCTIONAL CHARACTERISATION OF THE IMMUNE CELLS IN THE HUMAN BONE MARROW AND THE IMPACT OF AGEING, SENESCENCE AND CYTOMEGALOVIRUS

Erin Naismith¹, Birgit Weinberger¹, Luca Pangrazzi¹, Klemens Trieb², Beatrix Grubeck-Loebenstien¹

¹Institute of Biomedical Aging Research, University of Innsbruck, Innsbruck - Austria

²Primararzt für Orthopädie. Ärztlicher Direktor. Klinikum Wels, Wels - Austria

Purpose: It has been shown that many antigen experienced immune cells migrate back to the bone marrow (BM), where they can remain in bone marrow niches for an extended period of time. In this study a detailed phenotypical and functional characterisation of immune cells isolated from human bone marrow was analysed. Various cellular senescence markers were used to determine if the accumulation of highly differentiated CD8+ T cells in the aging bone marrow limits the possibility of the accumulation of other immune cells. Chronic infection such as Cytomegalovirus (CMV) was also considered in the analysis to determine if survival trends could be seen among long living immune cells.

Methods: Bone Marrow Mononuclear cells were isolated from human bone marrow samples using collagenase digestion and density gradient centrifugation. Surface and senescence phenotypes were characterised using flow cytometry. Cells were stimulated with PMA Ionomycin for Intracellular staining and cytokine production was also analysed using flow cytometry.

Results: Multiple correlations are seen between cell subsets found in the Aging bone marrow, as well as with age. The number of CD8+CD28- T cells which correlate positively with age, negatively correlates with IgM Memory B cells (CD27+IgD+CD19+), and positively correlates with CD4 Effector Memory cells (CD45RO+CCR7-). Several senescent phenotypes are more prevalent in CMV seropositive individuals, and there is a higher expression of inflammatory cytokines such as IFN γ and TNF α .

Discussion: From these preliminary data the concept of immunological bone marrow niches comprising of limited space suggests to affect the immune cell composition in the aging bone marrow. The accumulation of one cell type in the bone marrow, may limit the possibility of others being maintained. Further investigation would focus on where these limitations are set and whether the specificity of these long living memory cells effects their ability to migrate back to the bone marrow.

Conclusion: The accumulation of highly differentiated CD8+CD28- in the aging bone marrow has previously been shown, however the impact of this subpopulation on other cell subsets has not yet been described. The interactions of these subsets and the presence or absence of various differentiation and senescence markers may give us more information on the ability of the bone marrow to harbour long living immune cells, and whether there are limiting factors determining this possibility.

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P11.5

IMMUNE CHARACTERIZATION OF THE HBHA-SPECIFIC RESPONSE IN MTB-INFECTED PATIENTS WITH OR WITHOUT HIV INFECTION

Teresa Chiacchio¹, Giovanni Delogu², Valentina Vanini¹, Gilda Cuzzi¹, Flavio De Maio², Carmela Pinnetti³, Alessandro Sampaolesi³, Andrea Antinori³, Delia Goletti¹

¹Translational Research Unit, Department of Epidemiology and Preclinical Research, ³Department of Clinical and Clinical Research, National Institute for Infectious Diseases (INMI) "L. Spallanzani", Roma

²Institute of Microbiology, Catholic University of Sacred Heart, Roma

Purpose: It would be important to identify subjects at risk to develop tuberculosis (TB) disease. RD1-based Interferon- γ Release Assays (IGRAs) cannot distinguish latent from active TB disease. Conversely, a positive response to HBHA-based IGRAs, among TB-infected subjects, correlates with Mtb containment and low risk of TB progression. Aim of this study is to characterize HBHA-immune responses in HIV-infected and uninfected subjects with active TB or latent TB infection (LTBI).

Methods: 49 subjects were prospectively enrolled, 22 HIV-uninfected (13 TB, 9 LTBI) and 27 HIV-infected (12 HIV-TB, 15 HIV-LTBI). Whole blood and PBMC were stimulated with HBHA and RD1 antigens. IFN γ release was evaluated by ELISA whereas cytokine profile (IFN γ , TNF α , IL2) and phenotype (CD45RA, CD27) by FACS.

Results: Among LTBI individuals, HBHA stimulation induced IFN γ release in all the HIV-uninfected, while only 4/15 HIV-infected responded. Within the active TB, only 6/13 HIV-uninfected and 1/12 HIV-TB patients responded. Differently than what observed for RD1, the cytokine profile of HBHA-specific T cells evaluated by FACS showed that the CD4 T cells were mostly monofunctional. Conversely, CD8-specific T cells were mostly monofunctional for both HBHA and RD1 stimulations. The phenotype of HBHA-specific T cells showed a predominantly central memory (CM) and effector memory (EM) phenotype in CD4 T cells without differences among the groups. Differently, HBHA-specific CD8 T cells, showed mainly a CM and naive phenotype in LTBI group while TB, HIV-LTBI and HIV-TB groups were characterized by EM or terminally differentiated (T_{EMRA}) phenotypes.

Conclusions: These results may help to better define immunological correlates of protection from TB disease.

P11.7

Pbx-REGULATING-PROTEIN 1 (PREP1) AS A NOVEL TRANSCRIPTION FACTOR LINKING IMMUNITY AND METABOLISM

Deriggio Faicchia¹, Claudio Procaccini², Serena Cabaro³, Antonietta Liotti³, Francesco Oriente¹, Vincenzo Gigantino⁴, Sara Bruzzaniti⁵, Francesco Blasi⁶, Pietro Formisano¹, Gianni Marone¹, Francesco Beguinot¹, Giuseppe Matarese⁷

¹Dipartimento di Scienze Mediche Traslazionali, ²Laboratorio di Immunologia, Istituto di Endocrinologia e Oncologia Sperimentale, Consiglio Nazionale delle Ricerche (IEOS-CNR) c/o Dipartimento di Medicina Molecolare e Biotecnologie Mediche, ⁷Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università di Napoli "Federico II", Napoli

³URT 'Genomica Funzionale' Istituto di Endocrinologia ed Oncologia Sperimentale, 'G. Salvatore', Consiglio Nazionale delle Ricerche (IEOS-CNR), Napoli

⁴Unità di Patologia, Istituto Nazionale Tumori, IRCCS, Fondazione Pascale, Napoli

⁵IEOS CNR, Napoli

⁶IFOM, FIRC Institute of Molecular Oncology, IFOM-IEO Campus, Milano

Purpose: Prep1 is an homeodomain transcription factor, which plays a key role in early development, in the regulation of energy homeostasis and metabolism(1). Prep1 heterozygous mice (Prep1^{+/+}) are protected from diabetes(2). Given the link between metabolic alterations and immune system functions, this study aims at characterizing the role of Prep1 in the modulation of immune response, associated with protection from metabolic and immune-mediated disorders.

Methods: Immune-phenotyping and activation of immune cells have been made by cytofluorimetric analysis, cytokine levels by Luminescence technology, whereas proliferation and suppression assays have been performed by thymidine incorporation. The metabolic profile of CD4⁺ T cells has been assessed by extracellular flux analyzer and the intracellular biochemical events by western blotting.

Results: Prep1^{+/+} mice showed a reduced T cell proliferation, impaired expression of activation markers and decreased levels of pro-inflammatory cytokines, as compared to their littermate controls. These effects were secondary to a reduced activation of the mammalian-target of rapamycin (mTOR) pathway, cell growth arrest and an altered bioenergetic profile of CD4⁺ T cells. On the contrary, CD4⁺CD25^{hi}Foxp3⁺ regulatory T cells (Treg) from Prep1^{+/+} mice displayed a higher ex vivo proliferative capacity and increased suppressive activity, conferring to these mice protection from metabolic alterations and hepatic inflammation induced by high fat diet (HFD).

Discussion: Prep1 can represent a novel tool for potential therapeutic manipulation of immune system functions to control progression of metabolic disorders (such as obesity and type 2 diabetes) and immune-mediated diseases.

Conclusions: These observations unmask a previously unknown role of Prep1 in the regulation of T cell function and provide a rationale for further investigating Prep1 as a possible target for immune-mediated disorders.

References

1. Berthelsen. 1998.
2. Oriente et al. 2013.

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P11.8

THE INTESTINAL T CELL LINES AS A SENSITIVE BIOASSAY FOR VALIDATION OF GLUTEN DETOXYFIFYING STRATEGIES

Stefania Picascia¹, Alessandra Camarca², Gianfranco Mamone², Serena Vitale¹, Ingrid S Pultz³, Aldo Di Luccia⁴, Carmen Gianfrani¹

¹Institute of Protein Biochemistry-CNR, Napoli

²Institute of Food Sciences-CNR, Avellino

³Department of Biochemistry, University of Washington, Seattle, Washington

⁴Department of the Sciences of Agriculture, Food and Environment, University of Foggia, Foggia

Purpose: Celiac Disease (CD) is a T cell mediated immune disease triggered by gluten. Gut-derived T cell lines (TCLs) have been largely used to dissect CD pathogenesis. Aim of this study was to validate the use of gluten-reactive TCLs as a sensitive bioassay to assess the gluten detoxification procedures, in addition to classical immunoenzymatic G12 and R5 methods.

Methods: TCLs were established from gut biopsies of 5DQ2 celiacs by stimulating lymphocytes 3 times with gliadin and subsequently with PHA. Gliadins from two different wheat detoxifying strategies were in vitro assayed for immunogenicity. The first procedure used an endopeptidase (Kuma030) with high activity to degrade gliadins; in the second wet wheat kernels were treated by a short pulse in microwave (MTW). The gluten content was evaluated by R5 and G12 ELISA. Stimulatory activity of treated gliadins on TCLs was evaluated by detection of IFN- γ and cell proliferation.

Results: All TCLs produced high amount of IFN- γ or proliferated when exposed to wild type-gliadin. The treatment of gliadin with Kuma030 strongly reduced the T cell response in a dose-dependent manner, similarly to the reduction of peptide amount, as detected by G12. On the other side gliadins from MTW kernels, resulted gluten-free when evaluated by the R5, but retained the ability to stimulate TCLs, as showed by high INF- γ production.

Discussion: The discrepancy in the detection of gluten epitopes in MTW-treated wheat between R5-ELISA and T cell assay indicates that some detoxifying methods may induce a conformational protein modification or aggregation, leading to false negative results by R5-method. By contrast, TCLs are less sensitive to conformational antigen changes and may detect more epitopes than those detected by R5 method.

Conclusion: The use of gut TCLs is of crucial importance to evaluate the efficacy of gluten detoxification technologies and should be a mandatory pre-clinical assay.

Reference

1. Camarca et al. 2009.

P11.9

IL-10 PRODUCING CCR6+T-CELLS ARE A DISTINCT POPULATION OF B-HELPER T-CELLS THAT PLAY A PATHOGENIC ROLE IN SYSTEMIC LUPUS ERYTHEMATOSUS

Federica Facciotti¹, Paola Larghi², Nicola Gagliani³, Alessandra Penatti⁴, Moira Paroni², Barbara Haringer⁵, Ilko Kastir², Massimiliano Pagani², Sergio Abrignani², Pier Luigi Meroni⁴, Richard Flavell⁶, Jens Geginat²

¹European Institute of Oncology, Milano

²INGM-National Institute of Molecular Genetics "Romao ed Enrica Invernizzi", Milano

³Department of Medicine & Department of General, Visceral and Thoracic Surgery, University Medical Center Hamburg-Eppendorf, Hamburg - Germany

⁴ASST Istituto G. Pini, Milano

⁵DRFZ- German Rheumatology Research Institute, Berlin - Germany

⁶Department of Immunobiology, School of Medicine, Yale University, New Haven - USA

Purpose: IL-10 is a dual cytokine since it inhibits T cell responses, but is also a potent B-cell growth and differentiation factor [1, 2]. In Systemic Lupus Erythematosus IL-10 is pathogenic because it promotes auto-reactive B-cells. Here we characterize a population of IL-10+ T cells that express CCR6 and help B cell responses, suggesting that these cells play a pathogenic role in SLE.

Methods: We purified T cells from human samples and test their capability to perform B cell help in a mixed T-B co-culture. Reporter mice were used to monitor the presence of CCR6+IL-10+ cells in vivo.

RESULTS: We identified CCR6+ cells in human tonsils from healthy donors, and found that they are able to provide B cell help. We then compared them with Th17 cells, Tfh cells, and Tr1 cells, and found that these cells represent a distinct B helper T cell population. We then tested the presence of CCR6+ cells in vivo and found that they are able to provide B cell help in a partially IL-10 dependent manner. Finally, we checked the presence of CCR6+ in patients with active SLE and found that they are accumulated and contribute to the systemic IL-10 and autoantibody production, thus promoting disease progression.

Discussion: CCR6+IL-10+ T cells represent a distinct population which might be tolerogenic at the steady state but could be activated by recall antigens; we speculate that these cells could have a context dependent function, and, consistently, we show that IL-10-producing CCR6+T-cells from secondary lymphoid organs have potent B helper functions in humans and mice.

Conclusions: We identified a novel population of B-helper T-cells that might play a prominent pathogenic role in SLE. The monitoring of these cells might be a prognostic marker of SLE progression and/or activity, and targeting their B-helper functions is a promising therapeutic strategy.

References

1. Moore KW et al. Annu Rev Immunol 2001; 19: 683-765.
2. Sabat R et al. Cytokine Growth Factor Rev 2010; 21: 331-44.

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P11.10

THE INTRAFLAGELLAR TRANSPORT PROTEIN IFT20 IS REQUIRED FOR AUTOPHAGY IN T LYMPHOCYTES

Chiara Cassioli¹, Francesca Finetti¹, Anna Onnis¹, Valentina Cianfanelli², Francesco Ceconi³, Cosima Baldari¹

¹Department of Life Sciences, University of Siena, Siena

²Unit of Cell Stress and Survival, Danish Cancer Society Research Center, Copenhagen - Denmark

³Department of Biology, University of Roma 'Tor Vergata', Roma

Purpose: Autophagy is a catabolic process that delivers cytoplasmic material and damaged organelles to lysosomes for degradation. This process has emerged as central for lymphocyte homeostasis as well as for the generation of a productive immune response¹. Among the components of the intraflagellar transport system (IFT), recent studies have revealed a new role for IFT20 in autophagy in ciliated cells² beyond its well-established function in ciliogenesis. Since IFT20 acts as a crucial player in the orchestration of vesicular trafficking and cell activation in the non-ciliated T cell³, here we assessed the potential role of IFT20 in T cell autophagy.

Methods: We measured the autophagic flux in control and IFT20 knocked-down (IFT20KD) T cells by immunoblot detection of LC3-II. We then analysed the association of IFT20 with components of the Beclin-1/class III PI3K complex (PI3KC) as well as specific autophagy-related (ATG) proteins by co-immunoprecipitation experiments. Finally, we investigated the impact of IFT20 depletion on autolysosome generation and function by confocal microscopy and immunoblotting.

Results: We found that IFT20 interacts with components of Beclin1/PI3KC and is required for the assembly of the phagophore-initiating complex. IFT20 depletion resulted moreover in a defect in cargo degradation following autophagosome-lysosome fusion due to defective traffic of cathepsin D to lysosomes.

Discussion: We provide evidence that IFT20 is required for at least two steps of the autophagic process: i) nucleation of the autophagosomal membrane by participating in the assembly of Beclin-1/PI3KC and ii) cargo degradation by regulating cathepsin D delivery to lysosomes and thus indirectly impacting on autolysosome function.

Conclusions: Collectively, our results suggest a function for IFT20 as a novel multifunctional regulator of T cell autophagy.

References

1. Bronietzki et al. Immunol Cell Biol 2015.
2. Pampliega et al. Nature 2013.
3. Finetti et al. Nat Cell Biol 2009.

P12 - TUMOR IMMUNOLOGY

P12.1

MOUSE BREAST TUMOR GROWTH AND METASTASIS INHIBITION VIA IMMUNOTARGETING OF THE CANCER STEM CELL ANTIGEN xCT

Laura Conti¹, Stefania Lanzardo¹, Elisabetta Bolli¹, Valeria Rolih¹, Andrea Ballatore¹, Roberto Ruii¹, Gaetano Donofrio², John P O'Rourke³, Federica Pericle³, Federica Cavallo¹

¹Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center, University of Torino, Torino

²Department of Medical Veterinary Science, University of Parma, Parma

³Agilvax, Inc. 5901 Indian School Rd NE, Albuquerque - USA

Purpose: The several unsuccessful treatments in metastatic cancers might miss cancer stem cells (CSC), which play a critical role in cancer. The identification of oncoantigens (OA) expressed by CSC may provide new targets for cancer therapies [1].

Methods: A transcription profiling analysis of the ErbB2⁺ TUBO cell line cultured as epithelial cells or tumorspheres was performed. Integrating data obtained with meta-analyses of 7 human breast tumor data sets we identified xCT, a channel that supports glutathione synthesis, as a new CSC OA that was validated in vitro and in vivo (2). To set up immunotherapies targeting xCT, we used xCT plasmids or an approach based on bacteriophage MS2 virus-like particle (VLP) or Bovine herpesvirus 4 based (BoHV4) technologies. Using genetic approaches, we produced VLPs displaying different xCT extracellular domains (ECD) or BoHV4 coding for xCT full protein.

Results: xCT expression increases over tumorspheres passages and its silencing significantly reduces tumorsphere generation. In vivo immunotargeting of xCT slows established subcutaneous tumor growth and impairs pulmonary metastasis formation in mice challenged with syngeneic tumorsphere-derived cells. This effect depends on the generation of specific antibodies that alter CSC self-renewal and redox balance and is improved when combined with cytotoxic drugs.

Discussion: We developed new vaccines targeting a freshly identified breast CSC OA, xCT, whose inhibition strongly impairs mammary tumor development and metastases.

Conclusions: This study provides a new tool for the design of combined therapeutic approaches that efficaciously target both breast CSC and differentiated cancer cells, leading to both cancer treatment and prevention of metastases.

References

1. Pan Q, et al. Stem Cells 2015.
2. Lanzardo et al. Cancer Res 2016.

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P12.2

THE TREATMENT OF MALIGNANT MELANOMA WITH A CHIMERIC DNA VACCINE AGAINST CSPG4: AN EFFECTIVE WAY TO OVERCOME IMMUNE TOLERANCE IN DOGS AND HUMANS

Federica Riccardo¹, Elisabetta Bolli¹, Giuseppina Barutello¹, Valeria Rolih¹, Maddalena Arigoni¹, Sergio Occhipinti², Elena Quaglini¹, Stefania Lanzardo¹, Soldano Ferrone³, Paolo Buracco⁴, Federica Cavallo¹

¹Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center, University of Torino, Torino

²Center for Experimental Research and Medical Studies (CERMS), Città della Salute e della Scienza di Torino, Torino

³Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston - USA

⁴Department of Veterinary Sciences, University of Torino, Grugliasco - Italy

Purpose: Due to the many similarities with its human counterpart, canine (c) malignant melanoma (MM) offers an excellent opportunity for translational clinical investigations. The chondroitin sulfate proteoglycan (CSPG)4 is an attractive immunotherapy target for both human (Hu) and cMM. We have demonstrated the clinical efficacy of a DNA vaccine against Hu-CSPG4 in a veterinary trial [1, 2]. Nevertheless, some vaccinated dogs eventually died because of metastasis. To increase the efficacy of our approach, we employed a hybrid plasmid coding for chimeric CSPG4 protein, expected to be effective in both veterinary and human settings.

Methods: We generated a hybrid plasmid in part derived from the Hu- and in part from the dog (Do)-CSPG4 sequences. We tested the safety and immunogenicity of HuDo-CSPG4 intramuscular DNA vaccination followed by electroporation (electrovaccination) in mice, in dogs with stage II-III surgically resected CSPG4⁺ oral MM, and in a human setting.

Results: The chimeric HuDo-CSPG4 is immunogenic in mice. In dogs HuDo-CSPG4 electrovaccination causes no side effects and induces antibodies (Ab) against both Hu- and Do-CSPG4, whose mechanism of action is under investigation. Most importantly, HuDo-CSPG4 vaccine is effective in significantly increasing the survival of canine MM patients. Interestingly, data obtained in vitro with T cells from human healthy donors also suggest HuDo-CSPG4 is more immunogenic than Hu-CSPG4 plasmid.

Discussion: The employ of hybrid HuDo-CSPG4 plasmid is an effective way to break immune tolerance against the self antigen in dogs and humans.

Conclusions: Considering the anti-tumor efficacy of HuDo-CSPG4 vaccine in canine patients and the high translational power of comparative oncology studies, these results could be of impact for both veterinary and human clinical panorama.

References

1. Riccardo F, Iussich S et al. Clin Cancer Res 2014; 20: 3753-62.
2. Piras LA, Riccardo F et al. Veterinary and Comparative Oncology 2016.

P12.3

DISCOVERY OF NEW CHEMOTHERAPY-ASSOCIATED ANTIGENS INDUCED BY IMMUNOGENIC CELL DEATH IN LUNG ADENOCARCINOMA WITH A REVERSE TUMOR IMMUNOLOGY STRATEGY

Alessio Grimaldi¹, Chiara Focaccetti¹, Giovanna Peruzzi², Gabriella Del Bene³, Sheila Spada⁴, Flavia Longo³, Paola Nisticò⁴, Carmine Mancone⁵, Vincenzo Barnaba¹

¹Dipartimento di Medicina Interna e Specialità Mediche, Roma

²Istituto Italiano di Tecnologia CLNS Sapienza, Roma

³Laboratory of Immunology and Experimental Oncology, Roma

⁴Dipartimento di Medicina Molecolare, Roma

⁵Dipartimento di Biotecnologie Cellulari ed Ematologia, Roma

Purpose: Immunogenic cell death (ICD) of tumor cells induced by chemotherapeutic drugs plays a key role in tumor immunotherapy. In fact, ICD affects antigen presentation leading to an improved anti-tumor T-cell response. Here, we aim to i) identify new immunogenic antigens from lung adenocarcinoma (LA) cells that result from chemotherapy-induced ICD, ii) assess the role of new antigens in survival and immune response improvement.

Methods: A primary lung cancer cell line (Pt4N), isolated and characterized for the expression of the epithelial marker EpCAM and for sensitivity to Cisplatin (CDDP), was metabolically labeled for the Stable Isotope Labeling by Amino acids in Cell culture (SILAC) using light (L-Lys; L-Arg) and heavy (¹³C₆Lys; ¹³C₆¹⁵N₄Arg) media. Light labeled cell line was subsequently treated with CDDP (0,625μM, 72h) inducing apoptosis. Then, untreated and CDDP-treated cells were sorted into separated populations (viable, or early and late apoptotic): viable and late apoptotic fractions were submitted to nano-Liquid Chromatography Tandem Mass Spectrometry to identify the differentially expressed proteins.

Results: Using a SILAC proteomic approach we obtain a large number of potential targets in late apoptotic fraction, with their relative abundance compared to viable cells.

Discussion: Focusing on over-expressed proteins in apoptotic cells we assess their function and cancer correlation using specific bio-informatics tools. Finally, using LA patients-derived memory T cell we analyze the immunogenicity for the new tumor associated antigens (TAAs) validation.

Conclusions: The T-cell interrogation system represents a strategy of reverse tumor immunology: it allows both to validate ICD in terms of improvement of tumor-specific responses and to identify chemotherapy-induced immunogenic TAAs as new prognostic tumor biomarkers.

References

1. Paroli M. Int J Cancer 2013.
2. Montaldo C. Proteome Sci 2014.

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12.4

PANCREATIC CANCER-RELATED INFLAMMATION: IN VIVO SCREENING TO IDENTIFY KEY TARGETABLE MOLECULES

Cecilia Roux¹, Roberta Curto¹, Gianluca Mucciolo¹, Francesco Novelli¹, Tak W. Mak², Paola Cappello¹

¹Department of Molecular Biotechnology and Health Sciences, University of Torino, Centre for Experimental Research and Medical Studies, Azienda Universitaria Ospedaliera Città della Salute e della Scienza, Torino

²University Health Network, Campbell Family Institute for Breast Cancer Research, Toronto, ON - Canada

Pancreatic ductal adenocarcinoma (PDA) is a highly aggressive malignancy characterized by resistance to chemo and radiotherapy. Emerging results support the role of immune system in creating a PDA-prone environment.

Purpose: To identify new targetable surface molecules by an in vivo screening of inflammatory-related proteins driving PDA progression.

Methods: We forced the expression of inflammatory-related genes in PDA cells and obtained hundred clones that were orthotopically injected in syngeneic C57BL/6 mice. We purified RNA from grown tumors and analysed by quantitative-PCR which genes promoted mass formation. Those genes were individually validated in vivo. In vitro assays as viability, migration and invasion were performed to characterize their biological role.

Results: To gather which gene gave advantages in in vivo growth we used a murine PDA cell line with low ability to grow when orthotopically injected in a syngeneic pancreas or to form metastases when injected intravenously. A first pool of ten clones injected in parallel to parental PDA cells, allowed identifying three genes, which were able to give rise tumor masses. In vitro analysis confirmed higher viability and migration rate for some of these clones that may be responsible for in vivo behaviour.

Discussion: We have demonstrated that an in vivo gain-of-function screen is suitable to identify inflammatory-related genes that promote PDA growth. Of note, all selected genes code for surface molecules that might represent candidate target to which develop inhibitory antibodies. We previously demonstrated that DNA-based vaccination strategy targeting a PDA-associated antigen, namely alpha-enolase (ENO1), significantly prolongs survival in a PDA mouse model by eliciting an integrated humoral and cellular immune response.

Conclusions: To this end, passive immunotherapies combined with ENO1-vaccination might represent an effective strategy to target PDA-related inflammation and enhance anti-tumor response.

References

1. Cappello P et al. Int J Cancer 2009; 125: 639-48.
2. Cappello P et al. Gastroenterology 2013; 144: 1098-106.
3. Cappello P, Novelli F. Oncoimmunology 2013; 6: e24384.

P12.5

IDENTIFICATION OF CHEMOTHERAPY-INDUCED ANTIGENS SUITABLE FOR IMMUNOTHERAPY IN PANCREATIC CANCER PATIENTS

Sara Bulfamante¹, Giorgia Mandili¹, Moitza Principe¹, Emanuela Mazza¹, Laura Follia¹, Giulio Ferrero², Andrea Evangelista³, Paola Cappello⁴, Francesco Novelli⁴

¹Department of Molecular Biotechnology and Health Sciences,

²Computer Science Department, University of Torino, Torino

³Unit of Clinical Epidemiology, Hospital Città della Salute e della Scienza di Torino, Torino

⁴Department of Molecular Biotechnology and Health Sciences, University of Torino, Hospital Città della Salute e della Scienza di Torino, Center for Experimental Research and Medical Studies, Torino

Pancreatic ductal adenocarcinoma (PDA) is one of the most lethal cancer, both for lack of effective screening method and for resistance to chemotherapy (CTX) and radiotherapy. However, some chemotherapeutic agents, such as Gemcitabine (GEM) have an immune modulating effect and more immunogenic antigens can be induced by CTX and targeted by passive or active immunotherapy.

Purpose: To discover TAAs that might be selected for immunotherapy, antibody response in PDA patients' sera were analyzed before and after CTX. TAAs selected on the basis of their increased recognition after CTX were used to evaluated whether PDA patient autologous T cells have an increased TAAs specific response after chemotherapy.

Material and Method: Antibody response in sera of PDA patients, before and after CTX treatments, has been analyzed by Serological Proteome Analysis (SERPA) on 2-dimensional gel electrophoresis proteome map of CFPAC PDA cell line and the antigens recognized were identified by mass spectrometry. T cell proliferation was evaluated by ³H-Thymidine incorporation assay on patients' PBMC stimulated with TAAs.

Results: Several recognized antigens correlate with over-expressed genes in PDA. The increased antibody recognition of four of these antigens correlates with longer survival. The antigens recognized more frequently by patients have been selected for the analysis of T cell response. In most cases PDA patients' PBMC obtained from the draw blood after one or two cycle of CTX showed higher T cell proliferation than before CTX.

Discussion: Data indicated that in PDA patients CTX induces an increase of antibody and T cell response to a series of TAAs whose expression is up regulated in PDA.

Conclusions: The identification of these antigens forms a platform for ongoing immunological studies aimed to assess the ability of these TAAs, to induce specific helper and cytotoxic response to PDA.

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P12.6

EXPLOITING DNA VACCINATION AGAINST ROS1 AS AN IMMUNOTHERAPEUTIC WEAPON AGAINST NON SMALL CELL LUNG CANCER

Federica Riccardo¹, Giuseppina Barutello¹, Maddalena Arigoni¹, Laura Conti¹, Chiara Musiu¹, Dario Livio Longo², Raffaele Calogero¹, Marco Volante³, Mauro Papotti³, Federica Cavallo¹, Elena Quaglini¹

¹Department of Molecular Biotechnology and Health Sciences,

²Department of Chemistry IFM and Center for Molecular

Imaging, ³Department of Oncology, University of Torino, Torino

Purpose: To identify targetable oncoantigens expressed during non small cell lung cancer (NSCLC) development, we performed a gene expression profile analysis in Kras^{G12D} mice that develop NSCLC, mimicking several features observed in lung cancer patients [1]. Among the genes overexpressed in Kras^{G12D} mice, the tyrosine kinase receptor ROS1 was identified as a candidate to be further investigated for immunotherapeutic strategies.

Methods: Organs of 10, 20 and 30 week-old wild type (wt) and Kras^{G12D} mice were collected for the analysis. A ROS1⁺ cell line (KL-ROS1) was generated from a Kras^{G12D} mouse lung tumor. The efficacy of ROS1-immunotargeting was evaluated using a mouse or a human anti-ROS1 DNA vaccine. Finally, tumor infiltrating-lymphocyte (TIL) analysis was performed by flow cytometry in Kras^{G12D} mice at 10 and 30 weeks of age.

Results: ROS1 overexpression was detected in both primary lung tumors and metastasis from Kras^{G12D} mice. Interestingly, cancer stem cell (CSC) enriched-lung spheres, derived from KL-ROS1 cells, were also ROS1⁺. Anti-ROS1 DNA vaccination against both KL-ROS1 subcutaneously injected cells and spontaneous lung tumors was quite effective. However, to identify the potential immunosuppressive mechanism that could affect the success of the DNA vaccines, we evaluated the evolving TIL during lung cancer progression in Kras^{G12D} mice. A prominent CD3⁺ infiltration characterized the early stage of tumor progression while immunosuppressive cells dominated the late response.

Discussion: Its overexpression in lung tumors, in metastasis and in CSC-enriched lung spheres suggests that ROS1 could be involved in the early and late stages of NSCLC progression and metastatization, making it an even more interesting target.

Conclusion: The combination of anti-ROS vaccination with the modulation of the immunosuppressive microenvironment in the lung lesions could result in an effective strategy to fight against ROS1⁺ tumors.

Reference

1. Riccardo F et al. BMC Genomics 2014.

P12.7

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

Elena Lo Presti¹, Serena Meraviglia¹, Gabriele Pizzolato¹, Veronica Catalano¹, Giuseppe Cicero², Jean Jacques Fournie³, Francesco Dieli¹

¹Department of Biopathology and Medical Biotechnologies

(DIBIMED)-CLADIBIOR, ²Department of Surgical and Oncological Sciences, University of Palermo, Palermo

³Centre de Recherches en Cancérologie de Toulouse (CRCT), Toulouse - France

Purpose: We have analysed frequency, phenotype and functions of gd T cells infiltrating CRC and correlated levels of intratumoral gd T cells with any of the established clinicopathologic features described for CRC.

Methods: CRC-infiltrating gd T cells were from a cohort of 70 patients. Expression of surface and intracellular markers were determined by flow cytometry. Tissue microarray (TMA) and transcriptome analysis were performed on two independent cohorts consisting of 185 and 585 patients, respectively.

Results: The majority of gd T cells in both CRC and adjacent normal tissues expressed Vd1, but intratumoral gd did not exhibit a distinct prevalence and distribution of Vd1 and Vd2 subsets, compared to adjacent normal tissue. Most Vd1 in tumor tissues were effector memory phenotype, whereas intratumoral Vd2 had more heterogeneous phenotype and both Vd1 and Vd2 in CRC and adjacent normal tissues preferentially produced IFN γ , but very low IL-17. IFN γ production by both Vd1 and Vd2 was significantly reduced in tumor tissue. Culture supernatants from cancer stem cells, but not from cancer associated fibroblasts, inhibited in vitro proliferation and IFN γ production by gd, CD4 and CD8 T cells lines. TMA and transcriptome analyses revealed that patients without lymph node metastasis and containing abundant gd and IFN γ had significantly longer 5-year disease free survival rate.

Discussion: Phenotypic and functional analysis indicates that gd producing IFN γ are a major component of normal and CRC tissues, and that inhibitory molecules produced in tumor microenvironment have a profound effect on several components of T cell responses. Both TMA and transcriptome analysis demonstrate that abundant gd and IFN γ significantly correlate with longer 5-year disease free survival rate.

Conclusions: Our study highlights a complex interplay between the tumor microenvironment and gd T lymphocyte response as an important determinant of the final outcome of CRC patients.

Reference

1. Wu P et al. Immunity 2014.

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P12.8

$\gamma\delta$ T CELLS PRODUCING IL17 OR IFN- γ ARE RECRUITED TO THE TUMOR SITE IN SQUAMOUS CELL CANCER AND DIFFERENTIALLY CORRELATE TO THE TUMOR STAGE

Elena Lo Presti¹, Serena Meraviglia¹, Francesca Toia², Sebastiano Oieni², Simona Buccheri³, Alice Turdo⁴, Laura Maria Mangiapane⁴, Francesco Dieli¹

¹Department of Biopathology and Medical Biotechnologies (DIBIMED)-CLADIBIOR, ²Department of Surgical, Oncological and Oral Sciences, Plastic and Reconstructive Surgery,

⁴Department of Surgical and Oncological and Oral Sciences, Cellular and Molecular Pathophysiology Laboratory, University of Palermo. Palermo

³Department for the Treatment and Study of Abdominal Diseases and Transplantation, Mediterranean Institute for Transplantation and Advanced Specialized Therapies (ISMETT), Palermo

Purpose: We have assessed the frequencies of tumor-infiltrating and circulating gd cells and Tregs from squamous cell carcinoma (SCC) patients, and their correlation with progression or survival.

Methods: SCC-infiltrating and circulating gd were analysed in a cohort of 47 patients. Expression of surface and intracellular markers was determined by flow cytometry. Supernatants from cancer stem cells, cancer-associated fibroblasts and differentiated cancer cells were obtained from SCC patients and normal tissues (n = 10).

Results: Vd1 infiltrated SSC tissue at higher levels than normal skin, and PBMC of patients and healthy subjects, while Vd2 showed higher frequency in PBMC than in tissue, either in cancer patients than in healthy donors. Tumor-infiltrating gd preferentially showed an effector memory phenotype and made both IL17 and IFN γ . IL17-producing gd amongst TILs were higher in patients with advanced disease, while the levels of IFN γ -producing gd were higher in SCC patients at early stage of disease. Different cell types in tumor microenvironment produced chemokines capable to recruit circulating gd to the tumor site and cytokines capable to reprogram gd to IL17 production. Tregs were decreased in blood of SCC patients, but were increased in tumor compartment. Frequencies of infiltrating Vd2 and Tregs differently correlated with tumor stage and ROC curve analysis suggest that the Vd2/Treg ratio might be of diagnostic potential.

Discussion: Our findings demonstrate that gd cells home to the tumor site, displaying differential cytokine production at different stages of tumor growth. Analysis of the Vd2/Treg ratio suggests that a reciprocal relationship may exist between Tregs and Vd2 in vivo, which is largely influenced by specific tumor microenvironment.

Conclusions: Our study suggests that phenotype and function of $\gamma\delta$ are largely shaped by tumor microenvironment, thus differentially affecting patients prognosis.

References

1. Lo Presti E et al. Front Immunol 2014.
2. Clark RA et al. Blood 2007.

P12.9

FUNCTIONAL CHARACTERIZATION OF SPECIFIC IMMUNE RESPONSE AND COMPARISON OF ORAL AND INTESTINAL HUMAN MICROBIOTA IN PATIENTS WITH COLORECTAL CANCER

Edda Russo¹, Giovanni Bacci², Elena Niccolai¹, Antonio Taddei¹, Federica Ricci¹, Maria Novella Ringressi³, Carolina Chielini², Camilla Fagorzi², Rossella Borrelli¹, Paolo Bechi³, Alessio Mengoni², Renato Fani², Amedeo Amedei¹

¹Department of Clinical and Experimental Medicine,

³Department of Surgery and Translational Medicine, University of Firenze, Firenze, Italy, Firenze

²Department of Biology, University of Firenze, Sesto Fiorentino, Italy, Firenze

Purpose: Colorectal cancer (CRC) is the second leading cause of cancer-related deaths in Italy. The CRC etiology has been recently linked to the gut microbiota. However, CRC-associated microbiota and the correlation with immune response is still largely unexamined.

Methods: Bacterial signatures from saliva, stools, and CRC biopsies of 30 control subjects and 30 CRC patients were detected using Next-Generation Sequencing (NGS) approach. DNA was extracted from each sample and used for Illumina NGS and species-level analysis was performed. Tumor-infiltrating lymphocytes were isolated from CRC patients and subsequently cloned and characterized.

Results: NGS analysis showed significant differences in bacterial population composition between control and CRC groups. The bioinformatic analysis revealed that i) control and CRC patients had different stool microbiota compositions, ii) representatives of *Fusobacterium* sp. genus seem to exhibit an association with the CRC group. Immunological analysis revealed that the number of intratumoral T cells that have a regulatory profile (Tregs) or are anergic increases in CRC patients.

Discussion: We observed an enrichment of Bacteroidetes phylum in CRC patients, whereas Firmicutes were over-represented in healthy controls. Bacteroidetes are highly associated with colon cancer because consumption of red meat and a high-fat diet (Western diet) stimulates bile flow, which, in turn, specifically stimulates Bacteroidetes. Furthermore, immunological profile in CRC patients seems to be impaired because the increased Tregs are able to antagonize the anti-cancer role of effector T cells (primarily, Th1).

Conclusions: Gut microbial composition differs between control subjects and CRC patients, that also, showed an intratumoral immune response with regulatory profile. These data suggest that a mutual interplay between gut microbiota and host immune system can be related to CRC development.

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P12.10

INTERPLAY BETWEEN BONE MARROW ENDOTHELIAL CELLS AND CD8 T CELLS IN MULTIPLE MYELOMA

Patrizia Leone¹, Giuseppe Di Lernia¹, Antonio Solimando¹, Ilaria Saltarella¹, Roberto Ria¹, Paolo Ditunno², Vito Racanelli¹, Angelo Vacca¹

¹Department of Biomedical Sciences and Human Oncology, University of Bari Medical School, Bari

²Hematology Unit, Di Venere Hospital, Bari

Purpose: Bone marrow (BM) endothelial cells (EC) are in close contact with CD8 T cells that come and go across the permeable capillaries. We analyzed the antigen presenting capacities of EC from BM of patients with multiple myeloma (MM) and monoclonal gammopathy of undetermined significance (MGUS).

Methods: BM EC from MGUS and MM patients were analyzed and compared with regard to their frequency, expression of HLA I antigen processing-presenting molecules, capacity to present antigens to and activate CD8 T cells. Experimental procedures included flow cytometry, immunomagnetic isolation, culture and stimulation of cells, proliferation assays, immunostaining and microscopy.

Results: In both MGUS and MM patients, almost all BM EC express HLA class I and lymphocyte function-associated antigen 3 (LFA3) molecules, whereas between 10 and 25% express the costimulators CD80, CD86 and CD40, and the inducible co-stimulator ligand (ICOS-L) thus suggesting a semi-professional antigen presenting phenotype. The EC immune/standard proteasome subunit ratio is in favor of immunoproteasome subunits as would normally be in professional antigen presenting cells. BM EC can present HLA class I-restricted tumor antigens to and activate CD8 T cells. Tumor-specific autologous CD8 T cells are CD45RA⁺CCR7⁻ and produce more IL-10 (90%) and TGF- β (80%) than IFN- γ (10%).

Discussion: Tumor-specific effector CD8 T cells in BM of patients with MM are inefficient because of the concomitant presence of endothelial cell-reactive tumor-specific memory CD8 T cells producing IL-10 and TGF- β .

Conclusions: BM EC promote MGUS-MM progression through a novel mechanism of immune escape.

Reference

Di Rosa F et al. Front Immunol 2016; 7: 51.

P12.11

MONITORING OF MELANOMA CLINICAL PROGRESSION BY CIRCULATING NK AND T CELLS IMMUNOPROFILING

Costanza Maria Cristiani¹, Rossana Tallerico¹, Valeria Ventura², Mariaelena Capone³, Gabriele Madonna³, Domenico Mallardo³, Cinzia Garofalo¹, Elina Staaf⁴, Ester Simeone³, Antonio M. Grimaldi³, Genny del Zotto⁵, Elio Gulletta², Gennaro Ciliberto⁶, Alessandro Moretta⁷, Paolo A. Ascierto³, Ennio Carbone¹

¹Tumor Immunology and Immunopathology Laboratory, Department of Experimental and Clinical Medicine,

²Department of Health Sciences, University "Magna Græcia" of Catanzaro, Campus - Germaneto, Catanzaro

³Melanoma Cancer Immunotherapy and Innovative Therapy Unit, ⁴Scientific Directorate, Istituto Nazionale Tumori Fondazione "G. Pascale", Napoli

⁴Department of Microbiology, Cell and Tumorbiology (MTC), Karolinska Institutet, Stockholm - Sweden

⁵Istituto Giannina Gaslini, Genoa

⁷Department of Experimental Medicine and Center of Excellence for Biomedical Research, University of Genoa, Genoa

Purpose: Natural Killer (NK) cells recognize low HLA class I expression typical of melanoma cells (1). Peripheral blood frequencies of CD56^{bright} and CD56^{dim} NK cell subsets are subverted in stage III melanoma patients (2). This research aims to validate and identify additional changes in the NK cells repertoire characterizing the transition from the different stages of melanoma that can improve the patient's diagnosis.

Methods: 2 healthy donors (HDs) and 191 melanoma patients (MPs) were enrolled. PBLs were isolated by Ficoll gradient and analysed by multiparametric immunofluorescence staining. Smaller subsets of HDs and MPs were used for seric cytokines analysis and CD107a mobilization assay. Data were analysed both in multivariate mode by SIMCA software and in univariate mode by paired t-test, ANOVA or Kruskal-Wallis test. p-values $\leq$ 0.05 were considered statistically significant.

Results: Compared HDs, MPs show an increase of CXCR2 and NKG2D and a reduction in NKp46 frequencies in the NK^{dim} cell subset. Of notice, stage II MPs have a higher percentage of circulating NK cells and a lower percentage of T cells. Both stage II and III MPs show reduced CD57 frequency and NKp46 expression on the NK^{dim} cells, that correlates with lack of responsiveness to K562 cells pulsing.

Seric concentrations of MCP1 exceeded the physiological range in all the MPs, while only in stage IV MPs there are also higher concentration of IL-6, IL-8 and IL-15.

Discussion: We confirm that in MPs there is an expansion of circulating CD56^{dim} CXCR2⁺ cells and increased seric levels of cytokines known to be involved in melanoma metastatic spread (2). Surprisingly, stage IV MPs have more mature and responsive NK cells in the periphery, suggesting a lower capability of these cells to migrate at the tumor site.

Conclusions: Our study confirm that NK cell subsets with different activities are selectively expanded among the different melanoma stages.

References

1. Burke et al. Trend Immunol 2010; 31: 339-45.
2. Ali et al. Nat Commun 2014; 5: 5639.

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P12.12

NUCLEAR FACTOR OF ACTIVATED T CELLS (NFAT), CANCER-INITIATING CELLS AND CHEMORESISTANCE

Valeria Corliano¹, Matias Cristobal Soncini¹, Ivan Zanoni²,
Francesca Granucci¹

¹Department of Biotechnology and Bioscience, University of
Milano-Bicocca, Milano

²Harvard Medical School and Division of Gastroenterology,
Boston Children's Hospital, Boston - USA

Purpose: Recent findings unraveled the involvement of Nuclear factor of activated T cells (NFAT) in cancer. NFAT activation may have both direct effects on tumor cells and indirect effects on tumor microenvironment. In murine mammary tumor cell lines constitutively activated NFAT promotes proliferation and metastasis, two hallmarks of cancer initiating cells (CICs). NFAT gene targets include COX2 and mPGES-1, required for the prostaglandin (PG)E2 production. Interestingly, PGE2 production by tumor cells has been recently shown to be responsible for chemoresistance by promoting CICs proliferation and a pro-tumor microenvironment.

Methods: NFAT signaling pathway-deficient mouse cell lines were generated. Sphere formation assay was used to estimate the percentage of CICs present in the population of tumor cells. Proliferation and viability were assessed by cell cycle analysis. Tumor formation and metastases in vivo were evaluated by transplanting VIVIT-transfected tumor cells and their mock counterpart in Balb/c mice.

Results: In a murine mammary tumor cell line (4T1) we observed that the inhibition of NFAT decreases the percentage of Sca1+ cells and reduces the ability to generate tumorspheres. NFAT inhibition affects the cell cycle in sphere-forming condition, increasing the percentage of cells in G0/1 phase and reducing that in S phase. Preliminary in vivo experiments showed that NFAT inhibition does not alter tumor formation while it impairs the ability to metastasize.

Discussion: Our results in 4T1 cells suggest that NFAT may have an intrinsic role in regulating CICs maintenance. NFAT inhibition impairs cell cycle progression in sphere-forming condition while it has no effect on cells in adhesion condition. Preliminary in vivo experiments confirmed the in vitro data.

Conclusion: Understanding the role of NFAT signaling pathway that regulates tumor progression will provide novel perspective for future pharmacologic intervention against cancer.

P12.13

DARPin® MP0250, A NEW ANTIANGIOGENIC DRUG IN MULTIPLE MYELOMA: IN VITRO AND IN VIVO STUDIES

Luigia Rao¹, Kim De Veirman¹, Donato Giannico¹, Ilaria Saltarella¹, Vanessa Desantis¹, Ulrike Fielder²,
Andreas Harstrick², Domenico Ribatti³, Karin Vanderkerken⁴,
Angelo Vacca¹

¹Department of Biomedical Sciences and Human Oncology
(DIMO), Section of Internal Medicine and Clinical Oncology,
Policlinico, Bari

²Molecular Partners AG, Zürich-Schlieren, Zurich - Switzerland

³Department of Basic Medical Sciences, Neurosciences and
Sensory Organs, University of Bari, Bari

⁴Department of Hematology and Immunology, Myeloma Center
Brussels, Vrije Universiteit Brussel, Brussels - Belgium

Purpose: DARPin® MP0250 (Molecular Partners AG, Switzerland) belongs to a novel class of innovative molecules exploited in cancer therapy that simultaneously binds VEGF-A and HGF with high specificity. Considering the role of VEGF-A and HGF in MM associated angiogenesis (1,2) we investigated whether MP0250 exerts antiangiogenic effects in MM.

Methods: Endothelial cells from MM patients (MMECs) were treated with MP0250 and VEGFR2/cMET signaling was evaluated by western blotting. In vitro functional assays were performed (angiogenesis, wound healing, chemotaxis and adhesion). In vivo antiangiogenic potential of MP0250 was analyzed by chorioallantoic membrane (CAM) assay and by matrigel plug assay. Syngeneic 5T33MM mice were treated with MP0250 (4 mg/kg, every 72 hours, intraperitoneally), Bortezomib (0,6 mg/kg, twice a week, subcutaneously) or the combination (combo) for 21 days. Microvessel density and tumor load were measured.

Results and Discussion: The treatment of MMECs with MP0250 reduced VEGFR2 and cMET phosphorylation and affected the dependent intracellular signaling cascade. In vitro studies showed that MP0250 inhibited MMECs functions. It altered the structure of the capillary network compared to the treatment with anti-VEGFA or anti-HGF agents and strongly synergized with Bortezomib halting MM-associated angiogenesis. In vivo, MP0250 significantly reduced the number of newly formed capillaries in the CAM assay and in the matrigel plug assay. In 5T33MM mice MP0250 administered as single agent decreased the microvessel density without affecting tumor load, while in the combo group a reduction of tumor load was observed.

Conclusions: The study demonstrated the antiangiogenic potential of MP0250 and provides the basis for a novel targeted therapeutic approach in MM.

References

1. Vacca A et al. Blood 1999; 93: 3064-73.
2. Ferrucci A et al. Clin Cancer Res 2014; 20: 5796-807.

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P12.14 SYNERGISTIC EFFECT OF BORTEZOMIB AND CHLOROQUINE IN MULTIPLE MYELOMA TREATMENT

Giuseppe Di Lernia¹, Ilaria Saltarella¹, Patrizia Leone¹, Antonio Solimando¹, Roberto Ria¹, Vito Racanelli¹, Angelo Vacca¹

¹Department of Biomedical Science, Section of Internal Medicine, University of Bari "Aldo Moro" Medical School, Bari

Purpose: Multiple Myeloma (MM) is defined as a neoplastic disease characterized by uncontrolled proliferation of plasma cells that covers 1% of all malignant tumours and about 10-15% of all the haematological malignancies. In this scenario it is necessary the development of new biological targets in order to enhance the current therapeutic strategies, such as, for instance, the use of the proteasome inhibitor bortezomib (Bort)1. Autophagy is a catabolic process well conserved among all mammals that has the pivotal role of keeping cells homeostasis by clearing and recycling any damaged or useless parts of a cell2. Due to the ability of preventing the fusion between lysosomes and autophagosomes, Chloroquine (Cq) has been identified as one of the best inhibitor of autophagy3. In this study we investigate on the effects achieved by the combination of Bort and Cq on MM cell line RPMI-8226, on Human Umbilical Vein Endothelial Cell line (HUVEC) and on ECs isolated from patients with the Monoclonal Gammopathy of Undetermined Significance (MGECS) and MMECs in vitro.

Materials and Methods: Cells have been treated with Bort and Cq alone or in combination, for different time points and concentrations. Specific antibodies for LC3-II and p62 protein have been used to describe autophagy modulation by western blot. Moreover, induction of apoptosis has been studied with Annexin V:PE Apoptosis Detection Kit I. Cell viability was determined by CellTiter-Glo[®].

Results: 100uM and 4h treatment with Cq were selected as best conditions to achieve the blockade of autophagy in RPMI-8226, HUVEC, MGECS and MMECs. Bort treatment seems to increase the autophagic flux more in MMECs than MGECS. On the other side, Cq undoubtedly improves the Bort treatment in RPMI-8226.

Discussion: Introduction of autophagy in MM treatment could be a promising alternative although we need to clarify the role of this process in MM. Bort treatment seems to be enhanced by the inhibition of autophagy although further experiments will be done to clarify this theory.

References

1. Berenson A et al. Outcomes of multiple myeloma patients receiving bortezomib, lenalidomide, and carfilzomib. *Annals of Hematology* 2017; 96: 449-59, doi:10.1007/s00277-016-2889-2.
2. Jin M, Klionsky, DJ. Regulation of autophagy: modulation of the size and number of autophagosomes. *FEBS Letters* 2014; 588: 2457-63, doi:10.1016/j.febslet.2014.06.015.
3. Ye H et al. Chloroquine, an autophagy inhibitor, potentiates the radiosensitivity of glioma initiating cells by inhibiting autophagy and activating apoptosis. *BMC Neurology* 2016; 16: 178, doi:10.1186/s12883-016-0700-6.

P12.15 JAM-A ROLE AS A PROGNOSTIC FACTOR AND NEW THERAPEUTIC TARGET IN MULTIPLE MYELOMA

Antonio Giovanni Solimando¹, Andreas Brandl², Katharina Mattenheimer², Carolin Graf², Miriam Ritz², Anna Ruckdeschel², Thorsten Stühmer³, Martina Rudelius⁴, Maria Antonia Frassanito¹, Vanessa Desantis¹, Giuseppe Di Lernia¹, Andreas Rosenwald⁴, Franz Jakob⁵, Angelo Vacca¹, Hermann Einsele³, Andreas Beilhack²

¹Department of Biomedical Science, Section of Internal Medicine, University of Bari "Aldo Moro" Medical School, Bari

²Interdisciplinary Center for Clinical Research Laboratory,

³Division of Hematology and Oncology, Department of Internal Medicine II, University Hospital of Würzburg, Würzburg - Germany

⁴Institute of Pathology, University of Würzburg and Comprehensive Cancer Center Mainfranken, Würzburg - Germany, ⁵Orthopedic Center for Musculoskeletal Research, Orthopedic Department, University of Würzburg, Würzburg - Germany

Purpose: Our aim was to verify the hypothesis that the cell membrane protein Junctional adhesion molecule-A (JAM-A) may represent a novel target and a clinical biomarker in multiple myeloma (MM).

Methods: We evaluated JAM-A expression by real time PCR (RT-PCR), fluorescence-activated cell sorting (FACS) and immunofluorescence (IF) in MM cell lines and in 132 MM patients at different stages. We then measured the concentrations of soluble JAM-A from MM and healthy subjects sera by enzyme linked immune assay (ELISA). JAM-A functions were investigated using transient gene silencing (siRNA) and JAM-A related angiogenesis in MM with in vitro and in vivo approaches.

Results: We found elevated JAM-A levels in patient-derived plasma cells and endothelial cells (MMPCs and MMECs) compared to controls and a correlation with poor prognosis. Furthermore, we observed a significant increase of soluble JAM-A in the sera of MM patients compared to healthy subjects. In addition, MM cell lines showed high expression of both membrane and cytoplasmic JAM-A. Interestingly, inhibition of JAM-A using specific siRNA resulted in diminished tumorigenic potential. Remarkably, migration of MM cells was also impacted. Moreover, the JAM-A pathways supported PCs by regulating MMEC adhesion and angiogenesis.

Discussion: To overcome drug resistance and to improve clinical strategy, approaches directed to both MMPCs and bone marrow microenvironment are under investigation. Despite JAM-A is associated with invasion and metastasis in several cancers, the role of JAM-A in MM is unclear. Our findings suggest that JAM-A is a biomarker of malignancy in MM and that soluble plasma JAM-A may contribute to serum-based clinical stratification.

Conclusions: JAM-A dysregulation in MM is important in both targeted therapy and as clinical parameter for MM.

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P12.16

FUNCTIONAL CHARACTERIZATION OF EPIDERMAL GROWTH FACTOR RECEPTORS IN ENDOTHELIAL CELLS FROM PATIENTS WITH MULTIPLE MYELOMA

Luigia Rao¹, Donato Giannico¹, Ivana Catacchio¹, Angelo Vacca¹

¹Department of Biomedical Sciences and Human Oncology, University of Bari, Bari

Purpose: The dysregulation of the epidermal growth factor receptors (EGFRs) promotes angiogenesis in several solid tumors [1]. The study aims to investigate whether EGFRs may be a target of angiogenesis inhibition in multiple myeloma (MM).

Methods: The expression of EGFRs isoforms was evaluated on endothelial cells (ECs), purified from BM of both MM (MMECs) and Monoclonal Gammopathy of Undetermined Significance (MGECS) patients, by RT-PCR. EGFR's ligands (EGF, TGF α , HB-EGF) were investigated by RT-PCR and by ELISA on MMECs conditioned media (CM). The expression of EGFR1 was evaluated on MMECs treated with BM stromal cells (BMSCs) CM from active MM patient, or co-cultured with increasing ratios of RPMI8266 MM cells. In vitro angiogenic assays were performed treating MMECs with EGFR's ligands and after EGFR1 inhibition using Erlotinib and after EGFR1 silencing.

Results and Discussion: EGFR1 was the most expressed receptor isoforms among EGFRs and its expression significantly increased on MMECs compared to MGECS, suggesting EGFR1 correlation with MM associated-angiogenesis. EGFR1 expression on MMECs was significantly increased treating cells with BMSCs CM and by co-culturing MMECs with growing numbers of RPMI8266, with or without transwell. The EGFR's ligands were first investigated by RT-PCR to assess their expression in MMECs. Among them, HB-EGF secretion on CM was found to be higher compared to EGF and TGF α . Stimulation of MMECs with HB-EGF, EGF and TGF α improved MMECs angiogenic functions with variable potency. Instead, MMECs properties were halted by treatment with Erlotinib and by EGFR1 silencing, confirming the role of EGFR1 during angiogenesis.

Conclusions: We observed EGFR1 involvement in MM-associated angiogenesis. EGFR1 expression and activation may be dependent from the changes of the BM microenvironment during disease progression.

Reference

1. Arteaga C. Semin Oncol 2003; 30: 3-14.

P12.17

NEUTROPHIL PLASTICITY IN THYROID CANCER

Maria Rosaria Galdiero¹, Tiziana Lamsione¹, Stefania Loffredo¹, Anne Lise Ferrara¹, Francesco Borriello¹, Raffaella Iannone¹, Gilda Varricchi¹, Gianni Marone¹

¹Center for Basic and Clinical Immunology Research (CISI), Department of Translational Medical Sciences, University of Napoli Federico II, Napoli

Background: Neutrophil function has long been limited to the acute phase of inflammation and resistance against pathogens. Neutrophils are among the inflammatory cells infiltrating the tumors and recent studies placed them as key effector cells in the orchestration of the 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 cancer of the endocrine system. No studies are so far available investigating the role of neutrophils in TC.

Objective: The aim 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 TC cell lines TPC1 and 8505c (TC-CM). Neutrophil functions (e.g. chemotaxis, activation, 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 CXCL8/IL-8. Neutrophil survival induced by TC-CM was mediated by GM-CSF. In addition, TC-CM 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 factors (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.

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P12.18 STUDY OF THE T-CELL RESPONSE IN PATIENTS AFFECTED BY ADVANCED NON-SMALL CELL LUNG CANCER (NSCLC) TREATED WITH PD-1 INHIBITOR

Maria Caterina Rossi¹, Laura Maggi¹, Manuela Capone¹,
Alessio Mazzoni¹, Paolo Petreni², Veronica Santarasci¹,
Gianni Montaini¹, Beatrice Rossetti¹, Francesca Mazzoni²,
Francesco Di Costanzo¹, Francesco Liotta¹,
Enrico Maggi¹, Francesco Annunziato¹, Lorenzo Cosmi¹

¹Department of Experimental and Clinical Medicine and
DENOTHE Center, University of Firenze, Firenze

²Department of Medical Oncology, Careggi University Hospital,
Firenze

Purpose: Nivolumab is a fully human IgG4 monoclonal antibody that binds PD-1 with high affinity, blocking the interaction between PD-1 and its ligands. The inhibitory effect of PD-1 is accomplished through a dual mechanism, promoting apoptosis in antigen specific T-cells and reducing apoptosis in regulatory T cells. The aim of our study is to evaluate immunological change in immune response in particular of T cell subsets, occurring in NSCLC patients during PD-1 inhibitor treatments.

Methods: Immunological evaluations of the selected patients has been performed before starting with nivolumab therapy, and after 60 days of treatment. The following phenotypical and functional parameters have been studied in each patient: the frequencies of lymphocytes subpopulations in peripheral blood, their cytokines production profile after in vitro polyclonal stimulation and also the frequency of Treg cells; in addition, we have evaluated the expression of cytotoxic molecules or/and inhibitory molecules on lymphocytes subpopulations.

Results: Our preliminary data showed that patients treated with nivolumab have a clear enhancement of T-cell responses and cytokine production. The patients affected by NSCLC after treatment with nivolumab showed higher level in frequency of interferon gamma and TNF alfa CD8+ and CD4 + producing T cells and also higher level of perforin and granzyme expressing CD8+ and CD4+ T cells. Furthermore the frequency of Treg cells have been reduced.

Discussion: Our preliminary data suggest that nivolumab treatment could potentiate T-cell response in NSCLC, that could allow the immune system to fight the cancer

Conclusions: The inhibition of the interaction between PD-1 and its ligands may contribute to suggest new therapeutic strategies for cancer immunotherapy.

Reference

1. Ang YL et al. Onco Targets Ther 2016.

P12.19 ROLE OF PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR β/δ IN THE PATHOPHYSIOLOGY OF MULTIPLE MYELOMA

Donato Giannico¹, Luigia Rao², Giuseppe Fracchiolla³, Angelo Vacca²

¹Department of Biomedical Sciences and Human Oncology,

²Department of Pharmacy, Pharmaceutical Sciences, University of Bari, Bari

Introduction: Peroxisome Proliferator Activated Receptors (PPARs) are transcription factors implicated in cell differentiation and in various metabolic processes. Three isotypes (α , β/δ , γ) have been identified and are all expressed on endothelial cells (ECs). However the role of PPAR β/δ in multiple myeloma (MM) endothelial cells (MMECs) is unclear [1]. The project aims to elucidate whether PPAR β/δ may be involved in MM-associated angiogenesis.

Methods: ECs have been purified from bone marrow of patients with MM (MMECs) and Monoclonal gammopathy of undetermined significance (MGECS). PPAR β/δ expression was evaluated by RT-PCR and western blotting. MMECs were treated with GW501516 and GSK3787, a selective agonist and inhibitor respectively, and angiogenesis functional assays (spreading, migration, matrigel assay) were performed. MMECs were directly and indirectly co-cultured with MM cells (RPMI8266) using increasing ratios of RPMI8266 and PPAR β/δ expression and cell activation was evaluated.

Results: Expression of PPAR β/δ was higher on MMECs compared to MGECS. Its activation stimulated MMECs in vitro angiogenesis by mRNA over-expression of angiopoietin like-4 protein, elastin, fibronectin and collagen. Data were also confirmed by PPAR β/δ inhibition. Moreover co-cultures of MMECs with RPMI8266 at different cell ratios, in the absence or presence of transwell, stimulated the release of PGI2 by MMECs enhancing PPAR β/δ expression and activation.

Discussion and Conclusions: Direct and soluble factors-mediated interaction between MM cells and MMECs may stimulate PPAR β/δ over-expression and MMECs activation. This activation induces neo-vessel formation and provides stabilization of the capillary network. Thus PPAR β/δ seems to play an important role in MM associated-angiogenesis and its over-expression and activation on MMECs correlates with progression of disease.

Reference

1. Laura Piqueras et al. 2007; 27: 63-9.

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P12.20

EXOSOMES-MEDIATE CROSSTALK IN MULTIPLE MYELOMA PROGRESSION AND DRUG RESISTANCE

Lucia Di Marzo¹, Vanessa Desantis¹, Ilaria Saltarella¹, Aurelia Lamanuzzi¹, Marcella Arciuli², Tiziana Annese², Anna Gallone², Beatrice Nico², Angelo Vacca¹, Maria Antonia Frassanito¹

¹Department of Biomedical Sciences and Human Oncology,

²Department of Basic Medical Sciences, Neurosciences and Sense Organs, University of Bari Medical School, Bari

Purpose: Exosomes (EXOs) mediate local and systemic cell-to-cell communication and regulate cell behavior by transferring mRNA, miRNAs and proteins to recipient cells. Recently, we demonstrated that bone marrow (BM) cancer associated fibroblasts (CAFs) promote tumor progression and drug resistance (DR) in multiple myeloma (MM) (1-3). In this study, we analysed the effect of MM-derived EXOs on CAFs in MGUS to MM transition and, in turn, the effect of CAFs-derived EXOs on endothelial (ECs) and MM cells.

Methods: EXOs isolation was performed from conditioned medium (CM) of CAFs purified from BM aspirates of 8 active MM patients and from CM of cultured RPMI8226 and U266 MM cells. Electron microscopy (TEM), dual immunofluorescence-confocal laser-scanning microscopy, western blot (WB), flow cytometry (FC) and qRT-PCR studies were performed to evaluate the CAFs- and MM-derived EXOs phenotypes, their miRs content and their mutual effect.

Results: TEM analysis of CAFs- and MM-derived EXOs showed a vesicles population with heterogeneous aspect, 50-100 nm sized. WB analysis defined the expression of commonly used EXOs surface markers as CD63, Hsp70 for CAFs and CD63, Hsp70, Alix for MM cells. Confocal microscopy showed the ability both CAFs and MM cells to uptake respectively MM- and CAFs-derived EXOs labelled with fluorescent dyes. Functional studies showed that MM-derived EXOs induce a specific miRNA profile as overexpression of miR-27b-3p, -125b-5p, -214-3p and activated phenotype, as expression of FAP⁺ and α SMA⁺ antigens, in MGUS and MM CAFs. In turn, MM CAFs released EXOs containing miR-27b-3p, -125b-5p, -214-3p are swallowed by MM cells and ECs. Functional studies also showed that CAFs-derived EXOs are able to stimulate angiogenic ability in ECs and to induce bortezomib-resistance in MM cells.

Discussions and Conclusions: Overall results suggest an important exosomal crosstalk among tumor cells, CAFs and ECs which lead to BM microenvironmental modifications, favouring MM progression and DR.

References

1. Frassanito MA et al. Leukemia 2014; 28: 904-16.
2. Frassanito MA et al. Leukemia 2016; 30: 640-8.
3. Di Marzo L et al. Oncotarget 2016; 7: 60698-711.

P12.21

PANCREATIC CANCER AND INNOVATIVE TREATMENT: THE SEEMING PARADOX TO BLOCK THE IMMUNE RESPONSE

Elena Niccolai¹, Federica Ricci², Giulia Nannini², Antonio Taddei¹, Maria Novella Ringressi¹, Simona Rolla³, Fabio Cianchi¹, Paolo Bechi¹, Francesco Novelli⁴, Amedeo Amedei²

¹Department of Surgery and Translational Medicine,

²Department of Experimental and Clinical Medicine, University of Florence, Florence

³Centre for Experimental Research and Medical Studies (CERMS), ⁴Immunogenetics and Transplant Biology Service, Azienda Ospedaliera Città della Salute e della Scienza di Torino, Turin

Purpose: To investigate the role of IL-22 in the pancreatic cancer (PC) genesis.

Methods: We characterized the tumor-infiltrating T cells (TILs) isolated from 30 PC patients by flow cytofluorimetric analysis (FACS), ELISA tests and tumor cell co-cultures. In vivo analysis of IL-22+ T cells was performed by immunohistochemical (IHC) analysis of surgical specimens of PC tissue and by evaluation of blood IL22+ T cells isolated from the same patients.

Results: 40% of Tcc was able to produce IL-22. We isolated 74% of T helper (Th)-22 and 76% of T cytotoxic (Tc)-22 from the PC tissue and 26% of Th22 and 24% of Tc22 from the surrounding healthy mucosa. The IHC analysis confirmed that the number of CD3+ T-cells co-expressing IL-22 was higher in the neoplastic tissue. We found that the majority of IL-22 producing Tcc produce also IFN- γ (Th1) and that the cytotoxic effects of IFN- γ producing T cells on the human pancreatic tumor cell line L3.6pl was significantly reduced by the IL-22 presence. IL-22-producing Tcc positively correlated with TNM staging and metastases in PC patients. The percentage of Th22 cells in the peripheral blood of PC patients was significantly higher than healthy donors.

Discussion: Our ex vivo and in vivo analysis support the dual role of the anti-tumor immune system, suggesting the IL-22 as factor of PC progression. The intratumoral IL-22 levels were elevated in PC patients and Th1/Th22 cells are the major source of IL-22, that antagonizes their cytotoxic activity. Finally, the increased levels of Th22 positively correlated with TNM and poorer patient survival.

Conclusions: Monitoring Th22 levels could be a good diagnostic parameter and blocking IL-22 signaling may represent a viable method for new anti-PC therapies.

Reference

1. Niccolai et al. PMID: 26590104.

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P12.22

DECREASED TREG LEVEL IN BONE MARROW OF LOW RISK MYELODYSPLASTIC SYNDROME PATIENTS CORRELATES WITH THE OCCURRENCE OF CLONAL EXPANSION OF CITOTOXIC EFFECTORS

Angela Giovazzino¹, Valentina Rubino¹, Anna Teresa Palatucci², Stefania Leone³, Vlenia Pernice¹, Giuseppe Cerciello³, Fabrizio Pane³, Fiorella Alfinito³, Giuseppe Terrazzano², Giuseppina Ruggiero¹

¹Dipartimento di Scienze Mediche Traslazionali, ²Dipartimento di Medicina Clinica e Chirurgia, Università di Napoli Federico II, Napoli

³Dipartimento di Scienze, Università della Basilicata, Potenza
Università di Napoli Federico II, Napoli

Several data have been showing that immune-dependent mechanisms might be relevant for the selection, expansion and dominance of dysplastic clone/s in a subgroup of Myelodysplastic Syndrome (MDS) patients. To date, valuable criteria to identify such subgroup of patients are still lacking. We previously described that specific alterations of immune profile, as represented by low Treg level and high expression of CD54 on CD8 effectors in Bone Marrow (BM), allow the identification of a subgroup of MDS patients in which an immune-mediated pathogenesis of the disease might be inferred.

Purpose: This study aims to correlate tolerance control derangement in BM with pathological expansion of T cell effectors in MDS. Preliminary data, obtained in 26 Low Risk patients, confirm that Treg show a clustered distribution in BM; moreover, their quantitative defect correlates with the recruitment and the activation state of cytotoxic T cells.

Methods: To investigate the occurrence of antigen-dependent clonal expansion of T cell effectors, we analysed, by flow cytometry, TCR Vb repertoire in BM as compared with peripheral blood in Low Risk patients and healthy donors. The presence of preferential T cell expansion in BM, respect to peripheral blood, was also evaluated.

Results: Our preliminary data indicate that BM expansion of CD4 and CD8 T cells characterises a subgroup of Low Risk MDS patients. Moreover, the presence of BM clonal expansion of CD8, but not CD4 T cells, is significantly related with low Treg level (< 2% of BM lymphocytes) and activation of BM cytotoxic effectors

Discussion and Conclusion: These data suggest that BM Treg level may represent a valuable criterion to identify the subgroup of Low Risk MDS patients in which immune-mediated mechanisms are relevant for MDS pathogenesis. A more homogeneous grouping of patients will improve clinical management of the disease, hopefully allowing a more effective employment of innovative immune-modulating strategies in MDS.

P12.23

TUMOR-DERIVED MSCS INHIBITION OF T CELLS IS ENHANCED BY IFN- γ AND TNF- α NOT ONLY THROUGH IDO, BUT ALSO THROUGH IL4I1 AND PDL1 PATHWAY

Gianni Montaini¹, Alessio Mazzoni¹, Beatrice Rossetti¹, Manuela Capone¹, Maria Caterina Rossi¹, Veronica Santarlasci¹, Laura Maggi¹, Oreste Gallo², Lorenzo Cosmi^{1,3}, Francesco Liotta^{1,3}, Francesco Annunziato^{1,3}

¹Department of Experimental and Clinical Medicine and DENOTHE Center, University of Firenze, Firenze

²Department of Surgery and Translational Medicine, University of Firenze, Firenze

³Regenerative Medicine Unit and Immunology and Cellular Therapy Unit of Azienda Ospedaliera Careggi, Firenze

Purpose: Evaluate the immunomodulatory activity of tumor-derived Mesenchymal Stem Cells (MSCs)

Methods: Single cell suspensions from tumor and normal tissue specimens were obtained and then analyzed by flow cytometry to evaluate the frequencies of T effector cell subsets and T regulatory cells. Cells were seeded in plastic flasks for the isolation and expansion of MSCs. Expanded tumor-MSCs were tested for their ability to express and produce immunomodulatory molecules. Microarray assay was performed comparing unstimulated tumor-MSCs versus IFN- γ plus TNF- α stimulated tumor-MSCs. In addition, co-culture experiment was performed to evaluate tumor-MSCs immunosuppressive activity on T cells.

Results: The ex-vivo analysis showed an increased presence of Treg in tumoral tissue, moreover a high percentage of both CD4 and CD8 T cell produced IFN- γ and TNF- α . These cytokines were able to significantly increase (mainly when combined) MSC's immunomodulatory molecule expression, such as Indoleamine 2,3-dioxygenase (IDO), Interleukin 4 Induced 1 (IL4I1) and CD279L (PDL1). Furthermore, when co-cultured with CD4 or CD8 T cells, tumor-derived MSCs showed a strong inhibitory effect on T cells proliferation. This inhibitory effect was suppressed in presence of 1 methyl-tryptophan (1MT), an IDO inhibitor, and significantly reduced in presence of Catalase (IL4I1 inhibitor) and anti-PD1 mAb.

Discussion: These data suggest that tumor microenvironment is really dynamic, and that the immunoregulation in this environment is not only driven by Treg but also by stromal cells like MSCs. This regulation could be activated by the immune system itself, with a negative feedback for the immune response against the tumor.

Conclusion: MSCs inhibit CD4 and CD8 T cells proliferation, this inhibition is enhanced by IFN- γ and TNF- α . The leading pathways involved, besides the already described IDO, are also IL4I1 and PDL1 ones.

References

1. Liotta F. & al PMID: 25647013
2. Davies L.C. & al PMID: 27671847

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P12.24

COX-2 EXPRESSION DISCRIMINATES CANCER-PROMOTING FROM CANCER-INHIBITORY INFLAMMATION ACROSS A WIDE RANGE OF MALIGNANCIES

Eduardo Bonavita¹, Santiago Zelenay¹

¹Cancer Inflammation and Immunity Group, Cancer Research UK Manchester Institute, The University of Manchester, Manchester - United Kingdom

Purpose: Inflammation has emerged as major factor promoting cancer development and progression. Yet, tumour infiltration by certain inflammatory cells, such as cytotoxic T cells, is associated with good prognosis and clinical benefit following cancer therapy. Thus, inflammation can have "bad" tumour-promoting effects or "good" cancer-inhibitory ones. How different types of inflammation are established and manipulated by a growing tumour remains unclear.

Methods: Cyclooxygenase (COX)-2, commonly upregulated in numerous cancers, has been implicated in various aspects of malignant growth including proliferation, angiogenesis and invasion. Recently, we showed that COX-2 activity in cancer cells dominantly enables progressive tumour growth by fuelling tumour-promoting inflammation and suppressing T cell-dependent tumour control¹. In order to extend these findings to human cancer we interrogated and analysed publicly available datasets (e.g. TCGA, METABRIC).

Results: Analysis of a primary cutaneous melanoma dataset showed a marked conservation of the mouse COX-2 dependent signature in melanoma. High levels of COX-2 positively correlated with factors associated with tumour-promoting inflammation and negatively with classic anti-tumour immune pathways. Here, we extended this analysis to publically available datasets other cancer types. We found that COX-2 expression invariably associates with factors of cancer-promoting inflammation including IL-6, IL-8, IL-1 β or CXCL1 and in selected cases it inversely correlates with mediators of anti-tumour immunity such as interferons and factors involved in type I immunity. Moreover COX-2 "inflammatory signature" can predict patient outcome.

Conclusion: Our findings suggest that COX-2-inflammatory signature in cancer biopsies might constitute a useful biomarker of unresponsiveness to immunotherapies and patient survival.

Reference

1. Zelenay S et al. Cyclooxygenase-dependent tumor growth through evasion of immunity. *Cell* 2015; 162: 1257-70.

P12.25

DRUG-INDUCED SENESCENT MULTIPLE MYELOMA CELLS ELICIT NK CELL ACTIVATION AND PROLIFERATION BY DIRECT OR EXOSOME-MEDIATED IL-15 TRANS-PRESENTATION

Cristiana Borrelli¹, Biancamaria Ricci², Elisabetta Vulpis³, Alessandra Zingoni³, Marco Cippitelli³, Cinzia Fionda³, Maria Rosaria Ricciardi⁴, Maria Teresa Petrucci⁴, Alessandra Soriani³, Angela Santoni³

¹Department of Molecular Medicine, Center for Life Nano Science@Sapienza, Italian Institute of Technology, Sapienza University of Roma, Roma

²Department of Orthopedics, Washington University School of Medicine, St. Louis - USA

³Department of Molecular Medicine, Istituto Pasteur-Fondazione Cenci Bolognetti, Sapienza University of Roma, Roma

⁴Division of Hematology, Department of Cellular Biotechnologies and Hematology, Sapienza University of Roma, Roma

Purpose: Treatment of Multiple Myeloma (MM) cells with sub-lethal doses of doxorubicin (DOX) or melphalan (MEL) leads to senescence and results in immune activation of NK cells. We have evidence indicating that drug-treated MM cells display an increased expression of IL-15, a cytokine involved in NK cell activation and proliferation/maturation. Our aim was to demonstrate the role of IL-15 trans-presentation mediated by senescent MM cells on NK cell proliferation.

Methods: The senescent secretome was evaluated by using a cytokine/chemokine array and luminex technology. The expression of IL15 and IL15Ra was analyzed by qPCR and FACS analysis on MM cells and by western blot on MM-derived exosomes. NK cell proliferation was assessed through BrdU incorporation using flow cytometry.

Results: We found that IL-15 is expressed at mRNA and protein level both in MM cell lines and in malignant PCs derived from patients' aspirates, while the protein is undetectable in the conditioned supernatants. We also demonstrate that MM senescent cells display higher levels of the IL-15/IL-15Ra complex and release increased amount of exosomes expressing IL-15Ra and low level of IL-15. We found that both senescent MM cells and exosomes enhance primary NK cell activation and proliferation. The direct involvement of IL-15 was then determined by employing IL15 blocking Ab during the assays.

Discussion: The constitutive expression of IL-15 receptor and the autocrine production of IL-15 in MM cells have been proposed as a mechanism of tumor propagation. Conversely, our findings strengthen the importance of senescence-based anticancer therapies due to the ability of irreversible arrested senescent MM cells to promote NK cell proliferation by IL-15 trans-presentation, indeed enhancing the tumor immune-surveillance played by these immune cells.

Conclusion: Our data show that drug-induced senescent MM cells promote NK cell activation and proliferation by IL-15 trans-presentation.

Reference

- Soriani A et al. *Ji* 2014. (579) Tumor immunology



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P12.26

MODULATION OF CXCR3/CXCL10 AXIS AFFECTS MIGRATION OF NATURAL KILLER CELL POPULATION IN MULTIPLE MYELOMA

Valentina Bonanni¹, Elisabetta Vulpis¹, Helena Stabile¹, Maria Rosaria Ricciardi², Maria Teresa Petrucci², Angela Gismondi¹, Alessandra Zingoni¹, Angela Santoni³, Giovanni Bernardini⁴

¹Department of Molecular Medicine, ²Division of Hematology, Department of Cellular Biotechnologies and Hematology, Sapienza University, Roma

³IRCCS, NeuRomad, Pozzilli, IS; Department of Molecular Medicine, Sapienza University, Laboratory affiliated to Istituto Pasteur Italia-Fondazione Cenci Bolognetti, Roma

⁴Department of Molecular Medicine, Sapienza University; IRCCS, NeuRomad, Pozzilli, IS, Roma

Purpose: To investigate if alterations in CXCL10/CXCR3 axis are associated with Natural Killer (NK) cell dysfunction in multiple myeloma (MM) and can be targeted to improve NK cell-based immunotherapy.

Methods: CXCL10 expression in bone marrow (BM) and serum of MM patients was determined by ELISA. Analysis of chemokine and activating receptors on NK cells were evaluated by flow cytometry. In mouse studies, i) NK cells were purified from spleen of WT and CXCR3^{-/-} mice and treated using different in vitro activation/expansion protocols ii) activated NK cell migration was examined in vitro by transwell assay and in vivo by adoptive transfer into MM-bearing mice.

Results: High CXCL10 serum levels in MM patients were associated with accumulation of CD56^{high} and reduction of CD56^{low}CD16^{low} NK cells in BM and with lack of NKp30 up-regulation on CD56^{low}CD16^{high} NK cells. Regarding mouse studies, cytokine activation has no effects on in vitro migration to CXCL10, while migration to CXCL12 is reduced after IL-15 activation. Adoptive therapy with IL-15-activated NK cells was the most effective for tumor clearance but NK cell homing was decreased in BM of MM-bearing mice and was rescued by CXCR3 deficiency.

Discussion: Our results indicate that the relative proportion of NK cell subsets in the BM changes only in patients displaying high CXCL10 levels, with an enrichment of non-cytotoxic NK cell population. In this group of patients, up-regulation of NKp30 on BM CD56^{low} NK cells did not occur, suggesting ongoing NKp30-based mechanisms of tumor evasion. Such mechanisms may underlie the disturbed NK cell surveillance also in MM-bearing mice, where reduced NK cell localization and function to tumor site can be reverted by CXCR3 deficiency.

Conclusions: High CXCL10 serum levels are linked to altered NK cell status in BM of MM patients that may have consequences on NK cell anti-tumor function. This is supported by evidence in the mouse model, demonstrating that CXCR3/CXCL10 axis can be exploited in NK cell-based immunotherapy.

Reference

1. Ponzetta A et al. Cancer Res 2015; 75: 4766-77.

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P12.27

IMMUNE HOMEOSTASIS AND EARLY PET RESPONSE IN PEDIATRIC HODGKIN LYMPHOMA

Teresa Perillo¹, Paola Muggeo¹, Chiara Novielli¹, Rosamaria Daniele¹, Massimo Grassi¹, Vito Muggeo², Nicola Santoro¹

¹UOC Oncologia ed Ematologia Oncologica Pediatrica, Bari

²Dipartimento di Scienze Economiche e Statistiche, Università di Palermo, Palermo

Purpose: Pediatric Hodgkin Lymphoma (HL) have achieved more than 90% five- years event- free survival (EFS) rates. Nevertheless treatment is associated with high toxicity, especially radiotherapy-induced long-term sequelae. Therefore, current studies focus on reducing toxicity without impairment of the excellent survival rates. Early response to treatment evaluated by PET/TC performed after two therapy courses is highly predictive of final outcome and EFS in HL. We evaluated the possible correlation between biomarkers and immune homeostasis at HL diagnosis and the probability of early PET/TC response.

Methods: We retrospectively analyzed data from twenty-seven pediatric patients affected by HL enrolled in the AIEOP LH 2004 protocol. At diagnosis, for each patient, we recorded peripheral blood cell count, lymphocyte/monocyte and neutrophil/lymphocyte rates, erythrocyte sedimentation rate (ESR), ferritin, Natural killers, CD19, CD3/CD19 and CD4/CD19 rates, and we correlated to early PET/TC.

Results: Both eosinophil and CD19 B-cell count were directly related to the probability of early PET/TC negativity, whereas platelet count inversely related ($p < 0.05$). Logistic regression analysis confirmed the impact of CD19 absolute count ($> 300/\text{mmc}$) ($p = 0.085$) and platelet count ($\leq 420 \times 10^{-3}$) ($p = 0.0003$) on early PET response.

Conclusions: Our results strengthen the hypothesis that a decrease of B Lymphocyte absolute count may be related to lower chemosensitivity and thrombocytosis is a marker of worse response to treatment as well.

Reference

1. Faruggia P, Puccio G, Sala A et al. The prognostic value of biological markers in paediatric Hodgkin lymphoma. *Eur J Cancer* 2016; 52: 33-40.

P12.28

BACH1 (BTB AND CNC HOMOLOG 1), THE ONCOGENIC REGULATOR ENHANCES MIGRATION OF HT-29 COLON CANCER CELLS

Sadaf Davoudian¹, Neda Shajari², Shima Salehi², Behzad Mansoori², Ali Mohammadi², Behzad Baradaran²

¹Humanitas Clinical and Research Institute, Rozzano

²Immunology Research Center, Tabriz University of Medical Sciences, Tabriz - Iran

Purpose: In nearly all instances, development of distant metastasis is the major cause of colorectal cancer (CRC) related mortality. Today the regulator factors in initiation of CRC metastasis is less known. BACH1 is a transcriptional factor and it has recently been reported to participate in enhancement of metastasis in tumor cells [1, 2]. We were prompted to seek BACH1 role in metastasis of colon cancer cells.

Methods: To substantiate our purpose, we silenced BACH1 expression using siRNA in HT-29 colon cancer cells and confirmed the results by real-time PCR and western blotting analysis. Scratch-wound motility assays measured capacity of tumor cell migration of HT-29 cells before and after BACH1 silencing. Moreover, we assessed other metastasis-related genes such as CXCR4, MMP1, MMP9, and MMP13 by qRT-PCR to evaluate their relation in migration and metastasis of HT-29 colon cancer cells following BACH1 silencing.

Results: Quantitative RT-PCR and western blotting analysis demonstrated significant reduction in mRNA and protein expression level of BACH1 following siRNA knockdown in HT-29 colon cancer cells. The metastatic -related genes showed the meaningful low expression level by qRT-PCR following transfection. After BACH1 silencing the remarkable decrease in migration of HT-29 cells was observed.

Discussion and Conclusions: Together, our data indicate that the BACH1 specific siRNA effectively decline colorectal cells migration activity, therefore this study uncovered the probable role of BACH1 in colon cancer migration. BACH1 silencing holds the promising therapeutic molecular targeting for CRC treatment.

Key words: BACH1, colorectal, cancer, migration, siRNA.

References

1. Yun J et al. *The EMBO Journal* 2011; 30: 4500-14.
2. Liang Y et al. *Journal of Biological Chemistry* 2012; 287: 33533-44.

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P12.29
ABSTRACT WITHDRAWN

P12.30
ANTI-TUMOR IMMUNIZATION OF MOTHERS DELAYS
NEUROBLASTOMA DEVELOPMENT IN CANCER-PRONE
OFFSPRING

Giuseppina Barutello¹, Claudia Voena², Federica Riccardo¹,
Aline Réal¹, Roberto Chiarle³, Federica Cavallo¹

¹Department of Molecular Biotechnology and Health Sciences,
Molecular Biotechnology Center, Torino

²Department of Molecular Biotechnology and Health Sciences,
Center for Experimental Research and Medical Studies, Torino

³Department of Pathology, Children's Hospital Boston, Boston
- USA

Purpose: Neuroblastoma (NB), the most common cancer in infants [1], is frequently associated with mutations in the anaplastic lymphoma receptor tyrosine kinase (ALK) gene [2-3]. Since we demonstrated that maternal immunization (MI) against Her2-neu oncoantigen (neu) is effective in hampering tumor onset in offspring prone to develop neu-positive mammary cancer, because of the passive transfer of maternal immunity and immune-complexes to the pups, eliciting their active immunization [4], we hypothesize a successful application of MI approach against the ALK oncoantigen in NB.

Methods: We exploited a preclinical model of spontaneous NB driven by the overexpression of a mutated form of ALK (ALK^{F1174L}) and MYCN oncogene in neural crest-derived cells. Female mice hemizygous for MYCN oncogene underwent DNA electrovaccination with a prime-boost immunization schedule using a plasmid that codes for the extracellular and transmembrane domains of the human ALK protein (ALK-ECTM) or a control empty vector, prior to be mated with males hemizygous for ALK^{F1174L}. Magnetic Resonance Imaging technique has been exploited to determine the effect of anti-ALK MI in hampering NB progression in ALK^{F1174L}/MYCN double transgenic offspring born from ALK-ECTM or control mothers. Immunofluorescence, Western blot and cytofluorimetric analysis were performed in order to assess the immune response elicited by anti-ALK immunization in mothers and their offspring.

Results: A significant reduction of tumor growth kinetic, together with a significantly enhanced overall survival, were shown in ALK^{F1174L}/MYCN offspring born from ALK-ECTM mothers compared to control offspring. Moreover, we detected specific anti-ALK vaccine-induced IgG antibodies in the milk and sera of vaccinated mothers and in the sera of their offspring.

Discussion: The results so far achieved are consistent with our recent findings about the role of DNA MI against specific oncoantigens as a weapon to hamper cancer development in genetically predestinated offspring.

Conclusions: This kind of study can pave the way for the potential application of MI against an oncoantigen to prevent neonatal malignancies, having a substantial impact on clinical practice.

References

1. Mettling K. Neonatal Netw 2013.
2. Mosse YP. Nature 2008.
3. Chen Y. Nature 2008.
4. Barutello G. OncoImmunology 2015.

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P12.31

B CELLS IN CANCER IMMUNOLOGY: THE EFFECTS OF INTESTINAL CANCER MACROENVIRONMENT ON B CELL EFFECTOR AND REGULATORY FUNCTIONS

Francesca Mion¹, Stefania Vetrano², Silvia Tonon¹, Viviana Valeri¹, Andrea Piontini², Alessia Burocchi³, Luciana Petti⁴, Barbara Frossi¹, Alessandro Gulino⁵, Claudio Tripodo⁵, Mario P. Colombo³, Carlo E. Pucillo¹

¹Department of Medical Area - University of Udine, Udine

²Department of Biomedical Sciences - Humanitas University, Milano

³Molecular Immunology Unit, Department of Experimental Oncology and Molecular Medicine, Fondazione IRCCS Istituto Nazionale Tumori, Milano

⁴Department of Biotechnology and Translational Medicine, University of Milano, Milano

⁵Tumor Immunology Unit, Human Pathology Section, Department of Health Science, Palermo University School of Medicine, Palermo

Purpose: B cells are classically considered as positive regulators of the immune response, however the regulatory function of distinct B cell populations has been described. This study aimed at a comprehensive characterization of how the systemic tumor environment, or macroenvironment, affects the B cell arm of the immune system.

Methods: The effect of tumor onset on B cells was investigated in three models of intestinal cancer: the APC^{Min/+} mouse, that carries a mutation in the tumor suppressor gene adenomatous polyposis coli, the mouse model for colitis-related colon carcinogenesis based on the treatment with azoxymethane (AOM) and dextran sodium sulfate (DSS), and the CT-26 model in BALB/c, developed by the ectopic implantation of colonic carcinoma cells.

Results: The frequency of total B cells was assessed in different organs of healthy and tumor-bearing mice. A result common to all three models was the significant increase of the percentage of CD19⁺ cells in tumor draining lymph nodes (LN) while only the Apc^{Min/+} mouse presented a diminished frequency of these cells in the spleen and peritoneum. Differently from CT-26 tumor-bearing mice and AOM/DSS treated mice, Apc^{Min/+} mice presented a higher percentage of IL-10-producing B cells, in respect to the wild type (wt) control, in mesenteric and inguinal LN but not in the spleen where the percentage of these CD19⁺IL-10⁺ cells was significantly decreased. In addition, compared to the wt counterpart, B cells purified from the spleen of the Apc^{Min/+} mouse had a weaker response to IL-10-inducing signals, both in terms of IL-10 competence and release. Immunophenotypic analysis of the mature B cell populations of the spleen revealed a decreased percentage of marginal zone B cells in the Apc^{Min/+} condition which could explain the differences observed in the IL-10-producing B cell population in this organ. Finally, in the Apc^{Min/+} model, the tumor macroenvironment activated a differentiation route that led to the generation of IgA⁺ lymphocytes in the spleen.

Discussion: Our data reveal that the tumor macroenvironment that develops in the three analyzed models differently affect IL-10-producing Bregs. Following tumor onset, Apc^{Min/+} mice present an expanded IL-10-producing B cell population in tumor-draining LN and this could represent an immune escape mechanism of tumors. Conversely, tumor progression leads to a switch to IgA-producing plasma cells in the spleen.

P12.32

RAPAMYCIN IMPAIRS ANTITUMOR CD8⁺ T-CELL RESPONSES AND VACCINE-INDUCED TUMOR ERADICATION

Nada Chaoul¹, Catherine Fayolle¹, Belinda Desrues¹, Marine Oberkampff¹, Alexandre Tang¹, Daniel Ladant¹, Claude Leclerc¹

¹Institut Pasteur, Paris - France

Background: The metabolic sensor mTOR regulates growth and division in cancer cells, thereby tested as candidate anticancer target. However, mTOR inhibitors failed to produce useful clinical efficacy, leading to cancer stabilization rather than eradication in patients, potentially because mTOR is also critical for T cell differentiation and response, especially that of T cells implicated in immunosurveillance. Indeed, recent studies using rapamycin demonstrated the important role of mTOR in differentiation and induction of the CD8⁺ memory in T-cell responses associated with antitumor properties.

Purpose: We analyzed the effect of rapamycin treatment on anti-tumor vaccine-induced (i) tumor regression and (ii) tumor-specific immune responses.

Methods: We used the TC1 mouse model of human papilloma virus-16-induced cervical carcinoma, expressing the E7 oncoprotein, that we injected to naïve mice (day 0). Tumor-bearing mice were vaccinated (day 14) with an E7-carrying vaccine (CyaA-E7) that eradicates TC1 tumors. Mice received daily injections of rapamycin (750 or 75 µg/Kg/day) for 14 or 22 days. We evaluated tumor growth and the recruitment of myeloid-derived suppressor cells and lymphocytes, and more specifically T cell subsets to tumor using flow cytometry. We also assessed T-cell responses in vivo.

Results: In vaccinated mice, rapamycin induced a strong dose-dependent inhibition of the vaccine-induced: (i) tumor control, (ii) cytotoxic CD8⁺ T cell recruitment to tumor site and E7-specific cytotoxic T-cell responses and (iii) reduction of T regulatory cells and myeloid-derived suppressor cells infiltration.

Discussions: These results distinctly show that rapamycin favored tumor progression in our model.

Conclusions: Taken together, our results demonstrate that although rapamycin might inhibit tumor cell proliferation, it completely abolishes T-cell-mediated antitumor immune responses essential for the therapeutic efficacy of anti-cancer vaccines.

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P12.33

HIGH OX40 EXPRESSION IDENTIFIES FULLY ACTIVATED OVARIAN TUMOR-INFILTRATING Treg AND CORRELATES WITH REDUCED PFS

Alessia Burocchi¹, Roberta Zappasodi², Beatrice Belmonte³, Alessandro Gulino³, Claudia Bassani¹, Marina Bagnoli⁴, Delia Mezzanzanica⁴, Domenica Lorusso⁵, Francesco Raspagliesi⁵, Claudio Tripodo³, Massimo Di Nicola⁶, Mario Paolo Colombo¹

¹Molecular Immunology Unit, Department of Experimental Oncology and Molecular Medicine, ⁴Molecular Therapies Unit, Department of Experimental Oncology and Molecular Medicine, ⁵Department of Gynecologic Oncology, ⁶Department of Medical Oncology, Fondazione IRCCS Istituto Nazionale Tumori, Milano

²Ludwig Collaborative and Swim Across America Laboratory, Memorial Sloan Kettering Cancer Center, New York - USA

³Tumor Immunology Unit, Human Pathology Section, Department of Health Science, Palermo University School of Medicine, Palermo

Purpose: To investigate whether the expression of the costimulatory molecule OX40 on ovarian tumor infiltrating-Treg correlates with patients' outcome.

Methods: T cell immune-phenotypic analyses were performed by flow cytometry and immunohistochemistry.

Results: The frequency of CD4⁺CD25⁺Foxp3⁺ Treg was significantly increased in tumor microenvironment compared to ascites, patient's peripheral blood (P-PB) and healthy donor peripheral blood (PB). Otherwise the percentage of Treg was not significantly different among ascites, P-PB and PB. OX40 expression on Treg progressively increased from peripheral blood (both P-PB and PB) to ascites, reaching the highest expression on tumor-Treg. Intriguingly we noticed that patients could be classified in two groups according to low or high OX40 expression on tumor-Treg. Correlation of OX40 expression with patient's outcome revealed reduced PFS in patients with the highest expression of OX40 on tumor-Treg. To explain why high OX40-expressing Treg correlates with a reduced PFS the expression of several molecules involved in Treg suppressive activity was evaluated. Every patients' tumor-derived Treg were divided into OX40^{high} and OX40^{low} fractions to be further evaluated for the expression level of: Foxp3, CD25, ICOS, Ki-67, PD-1, CTLA-4 and Helios by flow cytometry. Results show that OX40^{high} Treg expressed all these markers at higher level than OX40^{low} Treg, suggesting a more activated/suppressive phenotype. In agreement CD45RA was expressed at lower level on OX40^{high} Treg than on OX40^{low} Treg.

Discussion: This research indicates that high OX40 expression on tumor-Treg may be considered as a negative prognostic marker in ovarian tumor. Nowadays ovarian cancer treatments remain poorly effective in controlling the high rate of disease relapse after primary medical intervention. The need of new treatment including check-point inhibitors should consider OX40-based immunotherapy as a necessary complement.

Conclusions: These data suggest that OX40 expression on Treg may identify a subset of highly suppressive cells whose high frequency correlates with reduced PFS of patients with ovarian cancer.

(622) Tumor immunology

P12.34

RE-EDUCATING ANTIGEN-PRESENTING CELLS: AN INTERFERON-GAMMA DELIVERY SYSTEM FOR ANTICANCER THERAPY

Flávia Castro¹, Marta L. Pinto¹, Andreia M. Silva¹, Mário A. Barbosa¹, Susana G. Santos¹, Raquel M. Gonçalves¹, Maria J. Oliveira¹

¹i3S - Instituto de Investigação e Inovação em Saúde, Porto - Portugal

Anti-cancer immune responses depend on the efficiency of tumour antigens presentation and co-stimulatory signals provided by antigen-presenting cells (APCs). However, it is reported that dendritic cells (DCs) present at the tumour site have an immunosuppressive profile, which limit activity of effector T cells and support tumor progression. Additionally, macrophages were described to promote tumour progression and negatively impact on responses to therapy. Thus, APCs are promising targets to generate therapeutic immunity against cancer [1, 2]. We focused on the potential of Chitosan/Poly(γ -glutamic acid) nanoparticles incorporating interferon-gamma to modulate tumor cellular immunity and, consequently to affect cancer-cell related activities. Accordingly, we developed an interferon-gamma delivery system that modulates macrophage and DCs phenotype, promoting T cell activation and counteracting in vitro colorectal cancer cell invasion. We are currently testing this strategy in an in vivo model in order to re-educate resident, and recently recruited, immune cells towards a pro-inflammatory and anti-tumour phenotype.

References

1. Mantovani A, Marchesi F, Malesci A, Laghi L, Allavena P. Tumour-associated macrophages as treatment targets in oncology. Nat Rev Clin Oncol 2017.
2. Tuettenberg A, Steinbrink K, Schuppan D. Myeloid cells as orchestrators of the tumor microenvironment: novel targets for nanoparticle cancer therapy. Nanomedicine (Lond) 2016;11: 2735-51.

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P12.35

A NEW MARINE-DERIVED SULFOGLYCOLIPID TRIGGERS DENDRITIC CELL ACTIVATION AND IMMUNE ADJUVANT RESPONSE

Giuse Barra¹, Emiliano Manzo², Carmen Gallo², Genoveffa Nuzzo², Adele Cutignano², Clementina Sansone³, Adriana Ianora³, Daniela Fenoglio⁴, Alessia Parodi⁴, Giuseppe Pasquale¹, Gilberto Filaci⁴, Antonio Leonardi⁵, Angelo Fontana², Raffaele De Palma¹

¹Dept of Clinical & Exp Med, C/o II Policlinico, Napoli

²Bio-Organic Chemistry Unit, IBC-CNR, Pozzuoli

³Stazione Zoologica "A. Dohrn", Napoli

⁴CEBR, Università di Genova, Genova

⁵Dept. of Molecular Medicine and Medical Biotechnology, Napoli

Dendritic Cells (DCs) recognize infectious non-self molecules and engage the adaptive immune system thereby initiating long lasting, antigen-specific responses. As such, the ability to activate DCs is considered a key tool to enhance the efficacy and quality of vaccination. Here we report a novel immunomodulatory sulfolipid named β -SQDG18 that prototypes a class of natural-derived glycolipids able to prime human DCs by a TLR2/TLR4-independent mechanism and trigger an efficient immune response in vivo. β -SQDG18 induces maturation of DC with upregulation of MHC II molecules and costimulatory proteins (CD83, CD86), as well as pro-inflammatory cytokines (IL-12 and INF- γ). Mice immunized with OVA associated to β -SQDG18 (1:500) produced a titer of anti-OVA Ig comparable to traditional adjuvants. In an experimental model of melanoma, vaccination of C57BL/6 mice by β -SQDG18-adjuvanted hgp10 peptide elicited a protective response with reduction of tumour growth and increase in survival.

P12.36

N6-ISOPENTENYLADENOSINE TRIGGERS ANTI-GLIOMA INNATE IMMUNE RESPONSE DEPENDENT ON THE P53 STATUS OF CELLS THROUGH THE INDUCTION OF ULPB2 AND MICA/B

Mario Abate¹, Chiara Laezza², Alba D'Alessandro³, Simona Pisanti¹, Roberta Ranieri¹, Maurizio Bifulco¹, Elena Ciaglia¹

¹Dept of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", Baronissi

²IEOS CNR, Napoli - Italy, ³Dept. of Pharmacy, University of Salerno, Fisciano

Purpose: In anticancer research, recently a novel facet of cytotoxic agents has been underpinned. Indeed, cancer cell stress induced by these therapeutics can promote antitumor immune response. The immunogenic potential of N6-isopentenyladenosine (iPA), an isoprenoid modified adenosine with a well established anticancer activity was then explored.

Methods: As expected, iPA was able to induce a significant upregulation of cell surface expression of NKG2D ligands on glioma cells in vitro and xenografted in vivo.

Results: Specifically suboptimal doses of iPA (0.1 and 1 μ M) control the selective upregulation of ULBP2 on p53wt-expressing U343MG and that of MICA/B on p53mut-expressing U251MG cells. This event made the glioblastoma cells a potent target for natural killer (NK) cell mediated recognition through a NKG2D restricted mechanism. The co-treatment of iPA-treated U343MG cells with pifithrin- α , a specific inhibitor of p53 activity, completely prevented the iPA action in restoring the immunogenicity of these cells in a p53-dependent manner. Furthermore, accordingly to the preferential recognition of senescent cells by NK cells, we found that iPA treatment was critical for glioma cells entry in premature senescence through the induction of S and G2/M phase arrest.

Discussion: Collectively iPA can display an immune-mediated antitumor activity engaging the innate immune system to fight cancer cells through a mechanism that is dependent upon p53 status which becomes a key molecular signature in dictating the different effect on ULBP2 and MICA/B induction by iPA but more generally by stress stimuli. The lack of lymphopenia as adverse side effect in glioma xenograft in vivo, fully increase the translational relevance of iPA.

Conclusions: Because different aspects of glioma biology have been separately targeted with very limited success, iPA capable of tumoral cell division control while making the glioma immunovisible to NK clearance may represent a hopeful alternative to other established chemotherapeutics approaches.

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IMMUNE-RELATED URINARY MOLECULES AS A DIAGNOSTIC AND PROGNOSTIC BIOMARKERS IN PROSTATIC CANCER

Sergio Occhipinti¹, Marianna Maniaci¹, Erica Lombardi², Paolo Gontero², Mirella Giovarelli¹

¹Department of Molecular Biotechnology and Health Sciences,

²Departement of Medical Sciences, University of Torino, Torino

Purpose: Prostate cancer (PCa) is the most common malignancy in men. Early diagnosed localized disease can be successfully cured by radical surgery or radiation. In developed countries prostate-specific antigen (PSA) screening is practiced. However PSA lacks both sensitivity and specificity to accurately detect patients at risk of prostate cancer.

Even before the appearance of clinical symptoms, immune responses against PCa are evidenced by intratumoral leukocyte infiltration and inflammatory pathway activation. Because the urinary tract is indeed very close with prostate, immune mediators produced by stromal cells and/or by PCa-infiltrating leukocytes can be detected in the urine and represent novel biomarkers for diagnosis and/or prognosis of PCa.

Methods: Blood and urine sample were collected from subjects that received indication for prostatic biopsies. The percentage of different leucocytes populations were evaluated in peripheral blood by flowcytometry. The levels of a panel of cytokines related to inflammation, immune suppression and angiogenesis were evaluated in urine by multiplex assay.

Results: We observed in the peripheral blood an increase of natural Treg cells expressing membrane-bound TGF β in patients with PCa (CP) at early stage compared to healthy subjects (HS). Moreover, in CP at advanced stage we observed an increase of IL-17+ CD4 T cells compared to HS. Analysis of molecules in urine showed that levels of 5 analytes related to Th17 subpopulation displayed a mild correlation with the presence of tumor. Combining values of different analytes, multivariate ROC curve analysis show that the values together strongly correlated with the presence of tumor.

Discussion: Taken together, these data suggest that Treg, but mainly Th17 CD4+ T cells may be involved in the development or progression of prostate cancer.

Conclusions: Urinary analysis of immune related molecules could be clinically useful in detecting already present neoplastic lesions.

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28-COLOR, 30 PARAMETER FLOW CYTOMETRY TO DISSECT THE COMPLEX HETEROGENEITY OF TUMOR INFILTRATING T CELLS IN HUMAN LUNG ADENOCARCINOMA

Jolanda Brummelman¹, Federico Colombo², Francesca Clemente², Federica De Paoli¹, Gianluca Rotta³,

Pierluigi Novellis², Giulia Veronesi², Alessandra Roberto¹, Enrico Lugli¹

¹Laboratory of Translational Immunology, Humanitas Clinical and Research Center, Rozzano

²Humanitas Clinical and Research Center, Rozzano

³BD Biosciences Italy, Milano

Purpose: The heterogeneity of tumor-infiltrating lymphocytes and their relative pro- or anti-tumor potential can only be addressed by more powerful single cell approaches.

Methods: By using antibodies conjugated to new dyes excited by 5 lasers mounted on the FACS Symphony A5 (BD Biosciences), we developed 28-color FACS to profile millions of single T cells from human lung adenocarcinomas.

Results: BUV, BV and BB dyes were brighter than many standard dyes regularly used in polychromatic FACS, thereby allowing extreme flexibility in panel development and better sensitivity in detecting dimly expressed proteins. Spreading Error (SE), resulting from errors in the measurement of fluorescence, but not compensation was the main determinant of panel success: indeed, dye combinations with >400% compensation but limited SE could be easily included, thus leading to 28-color combinations. Computational barcoding coupled to single cell PCA and t-SNE reduced dimensionality of the dataset and identified putative functional subsets in blood, tumor and non-tumoral portion of the lung from the same patient (n=16). In this way, we revealed that PD-1^{bright} exhausted CD8+, enriched at the tumor site, are mainly confined in the CD69+ tissue-resident memory fraction and are T-bet+HLA-DR+, while are nearly absent from the early-differentiated CCR7+ fraction. The latter, in turn, were recruited from the circulation. Notably, PD-1^{dim} CD8+ T cells, recently suggested to be specifically reinvigorated following anti-PD-1 therapy, were markedly different from PD-1^{bright} cells in terms of phenotype and transcription factor expression. Among CD4+, we identified tumor-specific subphenotypes whose abundance correlated positively with negative prognostic parameters, i.e. the Standard Uptake Volume (SUV) as obtained by PET scan.

Discussion: High-content single cell profiling and computational analysis identify tumor-specific phenotype with a putative role in pro- or anti-tumor immunity

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ROLE OF THE ATYPICAL CHEMOKINE RECEPTOR CCRL2 IN COLON CANCER

Tiziana Schioppa^{1,2}, Annalisa Del Prete^{1,2}, Francesca Sozio^{1,2}, Laura Melocchi², Mattia Bugatti², William Vermi², Silvano Sozzani^{1,2}

¹Humanitas Clinical and Research Center, Rozzano (MI)

²Department of Molecular and Translational Medicine, Section of Pathology, School of Medicine, University of Brescia, Brescia - Italy

Purpose: Colorectal cancer (CRC) is a leading cause of cancer death in Western countries, thus making of particular importance the understanding and dissection of the mechanisms underlying its onset and development. Recent studies demonstrated that infiltrating leukocytes do play a fundamental role in CRC. The recruitment of immune cells at the tumour site is orchestrated by chemokines and their receptors. CCRL2 is a non-signalin seven-transmembrane domain receptor structurally related to the family of the "atypical chemokine receptors". CCRL2 is expressed by immune cells and also by cells with barrier functions, such as endothelial and epithelial cells (1). The role of this receptor in tumour development is unknown and we want to investigate his function in models of CRC.

Methods: WT, CCRL2 deficient mice and VillinCre/CCRL2^{fl/fl} were treated with azoxymethane (AOM) 10 mg/Kg of body weight by intraperitoneal injection. After 7 days each group received DSS 2% for 7 days followed by 14 days of water for recovery. This schedule was repeated for 3 cycles. Mice were sacrificed at day 70. Colons were collected for histological analysis or for RNA extraction.

Results: Our preliminary results show that the CCRL2 deficient mice and VillinCre/CCRL2^{fl/fl} mice show an exacerbated phenotype with higher number of lesions and histology score. Of note, similar results were obtained using the genetic model of the APC^{Min/+} mice crossed with CCRL2 KO mice.

Discussion: The pathogenic mechanism underlying the observed phenotype in CCRL2 KO is under investigation but our preliminary data suggest a role of this receptor not only in the recruitment of leucocytes but also a the epithelial layer.

Conclusion: These results propose a new role for CCRL2 in the regulation of cancer related inflammation and tumor promotion. Therefore, CCRL2 might represent a new potential pharmacological target in colorectal cancer.

Reference

1. Del Prete A et al. European J of Immunology 2013.

P12.40

IDENTIFICATION OF A SUBSET OF HUMAN NATURAL KILLER CELLS EXPRESSING HIGH LEVELS OF PROGRAMMED DEATH 1: A PHENOTYPIC AND FUNCTIONAL CHARACTERIZATION

Silvia Pesce¹, Marco Greppi¹, Giovanna Tabellini², Fabio Rampinelli³, Silvia Parolini², Daniel Olive⁴, Lorenzo Moretta⁵, *Alessandro Moretta^{1,6}, *Emanuela Marcenaro^{1,6}

*Co-last authors: these authors contributed equally

¹Department of Experimental Medicine (DI.ME.S.), ⁴Center of Excellence for Biomedical Research (CEBR), University of Genoa, Genoa

²Department of Molecular and Translational Medicine, Brescia

³Department of Obstetrics and Gynecology, Spedali Civili of Brescia, Brescia

⁴CRCM, Equipe Immunité et Cancer, Inserm, U1068; Institut Paoli-Calmettes; Aix-Marseille Université, UM 105; CNRS, UMR7258, F-13009, Marseille, France

⁵Department of Immunology, IRCCS Bambino Gesù Children's Hospital, Rome

Background: PD-1 is an immunologic checkpoint that limits immune responses by delivering potent inhibitory signals to T cells on interaction with specific ligands expressed on tumor/virus-infected cells, thus contributing to immune escape mechanisms. Therapeutic PD-1 blockade has been shown to mediate tumor eradication with impressive clinical results. Little is known about the expression/function of PD-1 on human natural killer (NK) cells.

Objective: We sought to clarify whether human NK cells can express PD-1 and analyze their phenotypic/functional features. **Methods:** We performed multiparametric cytofluorimetric analysis of PD-1+ NK cells and their functional characterization using degranulation, cytokine production and proliferation assays.

Results: We provide unequivocal evidence that PD-1 is highly expressed (PD-1bright) on a NK cell subset detectable in the peripheral blood of approximately one fourth of healthy subjects. These donors are always serologically positive for human cytomegalovirus. PD-1 is expressed by CD56dim but not by CD56bright NK cells and is confined to fully mature NK cells characterized by the NKG2A-KIR+CD57+ phenotype. Proportions of PD-1bright NK cells were higher in the ascites of a cohort of ovarian-carcinoma patients suggesting their possible induction/expansion in tumor environments. Functional analysis revealed a reduced proliferative capability in response to cytokines, low degranulation and impaired cytokine production upon interaction with tumor targets.

Conclusions: We have identified and characterized a novel subpopulation of human NK cells expressing high levels of PD-1. These cells have the phenotypic characteristics of fully mature NK cells and are increased in ovarian-carcinoma patients. They display low proliferative responses and impaired anti-tumor activity that can be partially restored by antibody-mediated disruption of PD-1/PD-L interaction.