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ORAL COMMUNICATIONS

(437) Innate Immunity and Inflammation

Role of neutrophils in an imiquimod-induced mouse model of psoriasis

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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 Aldara^{TM}, 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 γδ 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

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inhibited γδ 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 γδ 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 γδ T cells is needed in order to define potential novel regulatory axis able to modulate disease pathogenesis.

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(443) Autoimmunity

T-cell proapoptotic and antifibrotic activity against autologous skin fibroblasts in vitro is associated with IL-17A axis modulation in systemic sclerosis

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Background. IL-17A has been implicated in the pathogenesis of systemic sclerosis (SSc). We previously showed that skewed peripheral blood mononuclear cells (PBMCs)

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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, CXCL1, 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 surnatants. In subset experiments, co-cultures were treated with either IL-17A or IL-17A *plus* anti-IL17 receptor A monoclonal antobodies (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. CXCL-11, 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 CTGFmRNA expression in co-cultured fibroblasts was reduced to 0.33 fold, 0.24 fold, and 0.31 fold, respectively ($p<0.05$). This 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 paly antifibrotic effects in autologous skin fibroblasts, and might be implicated in fibroblasts apoptosis.

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(444) Tumor immunology

Anti-PD1 therapy effects on T cell repertoire and functions in patients with NSLC cancer: a preliminary study to identify biomarkers of efficacy

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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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(449) Chronic lymphocytic leukemia

Integrated CLL scoring system had prognostic activity on young and early-stage chronic lymphocytic leukemia

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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 Padua University Hospital from 1989 to 2015, 420 had available data of FISH, IGVH 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, IGVH mutated and CD38<30%; high-risk, subjects with 17p or 11q deletion and/or IGVH 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

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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.

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(453) Tumor immunology

IL-18 receptor marks functional CD8 T cells in non-small cell lung cancer

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Purpose: IL-18 is a pro-inflammatory cytokine, produced in response to several pathogen- or damage-associated patterns[1]. 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) $PD\text{-}1^+$ 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 $IL\text{-}18R^+$ 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 response to IL-18. Indeed, *ex vivo* IL-18 treatment of mononuclear cells enriched from T increased the IFN-γ production, especially by the $IL\text{-}18R^+$ ($Tbet^+Eomes^+$) 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.

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(465) T cells

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

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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.

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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? and TNF in the bone marrow was higher in CMV seropositive donors. Lower IL7R and higher IL-2/IL-15Rb 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.

(469) Innate Immunity and Inflammation

Group V secreted phospholipase A2 mediates the production of angiogenic and anti-angiogenic factors from human neutrophils

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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 A_2 ($sPLA_2$) are mediators involved in inflammatory diseases and tumors. Human neutrophils (PMNs) are both a source and a target of $sPLA_2$s. These cells release group V $sPLA_2$ (hGV) and can be activated by $sPLA_2$s 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. $sPLA_2$ activity was measured by EIA.

PMNs constitutively express mRNAs for the proangiogenic molecules VEGF-A_{165}, VEGF-B_{167}, VEGF-B_{186}, and Ang1. mRNA for VEGF-A_{121}, VEGF-A_{189}, 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 of 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 $sPLA_2$s, 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 (anb3 and a4b1). 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.

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(470) Tumor immunology

MYCN is an immunosuppressive oncogene dampening the expression of ligands for Natural Killer cell-activating receptors in human high-risk neuroblastoma

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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.

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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.

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(483) Innate Immunity and Inflammation

PTX3 and factor H functionally cooperate in promoting phagocytosis and killing of Aspergillus fumigatus

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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

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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*.

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(487) Autoimmunity

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

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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 cytofluorimetry upon an *in vitro* stimulation with recombinant IL-7, IL-23 and IL-18.

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

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.

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(490) Autoimmunity

CD4+ Memory-stem T cells: novel players in Rheumatoid Arthritis

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Purpose Memory Stem T cells (T_{SCM}) are long living self-renewing T cells, which play a relevant role in immunological memory[1,2,3,4]. 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-TNFα 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-TNFα 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-TNFα

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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.

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(495) Cytokines and Chemokines

IL-21 promotes Granzyme B-dependent NK/plasmacytoid dendritic cell functional interaction in cutaneous lupus erythematosus

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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.

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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.

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(507) Autoimmunity

Development of a novel epitope-based diagnostic assay for systemic sclerosis

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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 (PDGFRα) peptide library used for epitope mapping of monoclonal anti-PDGFRα antibodies[1], and two smaller libraries containing: b) the top 20 PDGFRα conformational binders and 60 conformational and linear peptides of a cognate protein forming a complex with PDGFRα; c) the top cognate protein peptide binders, and 15 chimeric PDGFRα/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.

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(530) Tumor immunology

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

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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 ($FSP1^+/FAP^+/\alpha SMA^+$), with higher proliferative rate compared to monoclonal gammopathy of undetermined significance (MGUS) CAFs[1]. BM CAFs from bortezomib (bort)-resistant patients are resistant *in vitro* to the drug and prevent bort-induced apoptosis of co-cultured MM cells[2]. 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,

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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.

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(554) Allergy and anaphylaxis

INVESTIGATING WASP VENOM ALLERGY: A B CELL PROLIFERATION ASSAY

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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.

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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 carboxyflurescein diacetate succinidimyl 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.

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(562) Tumor immunology

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

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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 FceRIg chain and the ability to produce high amounts of IFNɣ 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 FceRIg chain, along with the priming for enhanced IFNɣ 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 exploitment of NK cell effector functions.

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(565) Neuroimmunology

Nasal treatment of Experimental Autoimmune Myasthenia Gravis with a recombinant fusion protein containing the acetylcholine receptor T cell-epitope 146-162

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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 IFNγ and IL17 pro-inflammatory mRNA, and with upregulation of TGFβ, IL10, FoxP3 transcripts in lymph nodes and spleens. IFNγ 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

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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.

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(580) B cells

Inhibition of Notch-mediated crosstalk between endothelial cells and plasmacells reduces angiogenesis in multiple myeloma patients

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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

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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 no 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 where 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 colture 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.

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(584) Immunodeficiencies

Immunodeficiency behind encephalopathy

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PURPOSE: Autoimmune lymphoproliferative syndrome (ALPS) is a disorder of abnormal lymphocyte homeostasis with elevated level of CD4- and CD8-negative T lymphocytes

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(termed double-negative T cell- DNT cells). Cinical 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 fullfilled 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 boli 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.

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(594) Innate Immunity and Inflammation

COMPLEMENT PROTEIN C1Q PRODUCTION IN MALIGNANT PLEURAL MESOTHELIOMA

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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 express 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.

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

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(595) Immunodeficiencies

Analysis of the molecular mechanisms of C1-inhibitor deficiency induced angioedema

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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.

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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.

(607) Immunodeficiencies

CD8+CD28-CD127loCD39+ Treg Expansion: a New Pathogenic Mechanism for HIV Infection?

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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-CD127loCD39+ 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-CD127lowCD39+ T regulatory cells. These cells have antigen specificity against HIV proteins, suggesting their origin from HIV-specific T lymphocytes. Their frequency post

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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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(636) B cells

Drugs-loaded nanoparticles: a new approach for the treatment of B-cell malignancies

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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 refractive 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

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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.

(640) T cells

T cell costimulation blockade blunts pressure overload-induced heart failure

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PURPOSE Heart failure (HF) is a leading cause of mortality. Inflammation is implicated in HF, yet clinical trials targeting pro-inflammatory cytokines in HF were unsuccessful, possibly due to redundant functions of individual cytokines. We attempted to identify and exploit better targets in cardiac inflammation so as to enable an immunotherapy of HF.
METHODS We utilized the gold-standard mouse model of HF, as well as biopsies from patients with different forms of HF.
RESULTS We linked T cells with HF development in a mouse model of pathological cardiac hypertrophy and in human HF patients. We then proceeded to inhibit T cell function *in vivo.* T cell costimulation blockade, through FDA-approved rheumatoid arthritis drug abatacept, led to highly significant delay in progression and decreased severity of cardiac dysfunction in the mouse HF model. The therapeutic effect occurred via inhibition of activation and cardiac infiltration of T cells and macrophages, leading to reduced cardiomyocyte death. Treatment also induced production of anti-inflammatory cytokine interleukin-10 (IL-10). IL-10-deficient mice were refractive to treatment, whilst protection could be rescued by transfer of IL-10-sufficient B cells.

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DISCUSSION AND CONCLUSIONS These results suggest that T cell costimulation blockade could be therapeutically exploited as a possible HF treatment.

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(641) Autoimmunity

Targeted nanoparticles-based diagnosis and treatment of Rheumatoid Arthritis

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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

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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 BNPs 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 BNPs were able to efficiently and selectively delivery 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.

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POSTERS

(445) Innate Immunity and Inflammation

Role of MyD88-signaling in the imiquimod-induced mouse model of psoriasis: focus on innate myeloid cells

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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 (Aldara™) (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-independepent 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 γδ T cell

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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.

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(460) Tumor immunology

Exploiting DNA vaccination against ROS1 as an immunotherapeutic weapon against Non Small Cell Lung Cancer

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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

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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.

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(496) Mucosal immunity

Immunogenicity of a bivalent adjuvanted glycoconjugate vaccine against Salmonella Typhimurium and Salmonella Enteritidis

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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-CRM_{197}) and *S.* Enteritidis (O:9-CRM_{197}) conjugate vaccines (1,2), or with bivalent (O:4,5-CRM_{197} + O:9-CRM_{197}) 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.

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(503) Neuroimmunology

Dysregulation of Repressor Element 1-Silencing Transcription factor in a mouse model of multiple sclerosis

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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 unexpectly 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

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REST4 affects the anomalous expression of REST in EAE at specific stages and in particular areas remains to be established.

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(506) Tumor immunology

Neutrophil plasticity in thyroid cancer

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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

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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-coltured 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.

(509) Immunodeficiencies

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

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Common Variable Immune Disorders (CVID) is a heterogonous group of primary immunodeficiency that encompass several immune dysregulations in adaptive and innate compartment of immunity. CVID is characterized by hypogammaglobulinemia, which leads

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to recurrent bacterial infections, autoimmunity, cancer and impaired specific antibody response to vaccines [1–3].

PURPHOSE: 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.

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(510) Innate Immunity and Inflammation

Role of hypoxia and the triggering receptor expressed on myeloid cells in human macrophage polarization

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Purpose Macrophages (Mf) are a major component of the leukocyte infiltrate at sites of inflammation and tumor growth. Mf can undergo diverse forms of activation in response to environmental factors, polarizing into specialized functional subsets[1]. A hallmark of the pathologic environment is represented by hypoxia[1]. Little is known about the impact of the hypoxic environment on Mf 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 Mf 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% O_2) or hypoxia (1% O_2) and for additional 24 hr with LPS (for M1 polarization) or IL4 (for M2 polarization) and then phenotypically and functionally characterized.

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Results Hypoxia decreased Mf 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)-1[2] was induced in Mf and its engagement imparted a proinflammatory M1-skewed phenotype to M2-polarized Mf. Mf infiltrating the inflamed hypoxic joints of children with Juvenile Idiopatic Arthritis[2] express TREM-1 and are predominantly polarized towards a M1 proinflammatory phenotype.

Discussion We demonstrated that hypoxia exerts M2-polarizing effects on Mf and identified TREM-1 as a marker of hypoxic Mf 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 Mf polarization and point to a role of TREM-1 in JIA pathogenesis.

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(516) Neuroimmunology

Differential use of the hydroxycarboxylic acid receptor-2 pathways triggered by monomethyl fumarate in different cells

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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-κB via the AMPK/Sirt1 axis. Increasing evidence associates signaling through HCAR2 in

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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-kB 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.

(518) Immunodeficiencies

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

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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].

PURPHOSE: 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 Ca^{2+} 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. Ca^{2+} chelation did not affect the phagocytosis while it strongly reduced monocytes and PMN oxidative burst. Moreover, we observed an efficient Ca^{2+}-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 Ca^{2+} mobilization [3]. Alternative ways for Ca^{2+} mobilization might bypass the lack of BTK. The efficient Ca^{2+}-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].

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(525) Neuroimmunology

A possible role for nerve glial antigen 2 in dendritic cell activation

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We recently showed that experimental autoimmune encephalomyelitis (EAE) induced with myelin oligodendrocyte glycoprotein (MOG) in nerve/glial-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/IFNγ. IL-12 in

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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.

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(539) Allergy and anaphylaxis

FENNEL (FOENICULUM VULGARE) ALLERGY

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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

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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.

(540) Neuroimmunology

Exploring the role of microRNAs in the intra-thymic pathogenesis of myasthenia gravis

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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

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the autosensitisation 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 controls[2]. 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.
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(542) Neuroimmunology

An optimized protocol of differential centrifugation isolates distinct microvesicle subpopulations from myeloid cells

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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.

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(547) Autoimmunity

Rituximab induces a reduction of Lymphocyte CD3+ activated in patients with autoimmune diseases

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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/m^2. 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

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(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.

(548) Autoimmunity

Flogosis and Heart involvement in Large Vessel Vasculitis

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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/m^2) and wall thickened (3.96±0.83 mm). Aortic valve was regurgitant (AR) in 8.
Framingham score was 21.69±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.

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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 thickning. Uric acid appear related to vasa remodeling.

(551) Neuroimmunology

Ataxic sensory neuronopathy as sentinel symptom of primary Sjögren's syndrome: a case report

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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.

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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, pseudoathetosic 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.

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(572) Allergy and anaphylaxis

Allergy to lipid transfer protein: genetic basis

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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.
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(575) Innate Immunity and Inflammation

Pre-eclampsia is associated with defective production of C1q by invasive trophoblast

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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 cell[1] and promoting trophoblast interstitial invasion[2]. 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

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comparison to non pathological placentae. In vitro studies on trophoblast cells demonstrated that the expression of MMP-12, a marker of vascular remodelling[3], 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.

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(582) Innate Immunity and Inflammation

Genetic Variation in Autophagy-Related Genes Influences the Risk and Phenotype of Buruli Ulcer

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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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(591) B cells

Inhibition of mammalian target of Rapamycin (mTOR) through the dual mTOR inhibitor PP242 as antiangiogenic strategy in multiple myeloma

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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 (MGECs) 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

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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 MGECs 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 synergyed with bortezomib and lenalidomide reducing network of capillary-like structures on Matrigel®.

Discussion mTORC2 is manly 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.

(593) Mucosal immunity

Regulation of DNAM-1 family receptors and their ligands in physiological and pathological gut mucosa infiltrate and epithelium T cell populations

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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

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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-b, 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-b 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.

(601) Immunodeficiencies

IgM and IgA anti-pneumococcal capsular polysaccharides as prognostic tool for Common Variable Immunodeficiency: a longitudinal study

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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.

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(608) Tumor immunology

Anti-tumor immunization of mothers delays neuroblastoma development in cancer-prone offspring

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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

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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.
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(614) Innate Immunity and Inflammation

Role of the atypical receptor CCRL2 in the pathogenesis of Rheumatoid Arthritis

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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 subsets[1]. CCRL2 was shown to be one of the most upregulated genes in neutrophils from the synovial fluid of rheumatoid arthritis patients[2]. 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 100µg type II chicken collagen, Serum-Transfer Induced Arthritis by i.p. injection of 150µl 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 moAb 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.

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(623) Autoimmunity

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

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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, SLEDAY-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 xerophtalmia 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 xerophtalmia developed. Reduction of C3 (<90 mg/dl) was found in

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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 xerophtalmia and possible evolution to secondary Sjögren's syndrome.

(624) Autoimmunity

Immunological tolerance mechanisms controlling human autoreactive CD8+ T cells in healthy and autoimmune conditions

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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-a (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.

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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.

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(631) Immunodeficiencies

CLEARANCE OF REACTIVATED HIV-1 RESERVOIRS BY NATURAL KILLER CELLS

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PURPOSE: Pilot HIV-1 eradication studies showed that immune responses of patients with antiretroviral therapy are ineffective at clearing viral reservoirs reactivated through latency-reversing agents (LRAs). We hypothesize that, due to the function of LRAs and to viral activities previously reported, $CD4^+$ T cells harboring reactivated HIV-1 should express ligands for the activating NKG2D receptor (*i.e.* MICA/B and ULBP1-6 proteins) and become targets for NKG2D-mediated lysis by natural killer (NK) cells.

METHODS: Latently HIV-1-infected $CD4^+$ T cells were treated with LRAs then analyzed for the expression of NKG2D ligands (NKG2DLs) and susceptibility to lysis by NK cells.

RESULTS: We found that ULBP2 is consistently up-regulated on those primary $CD4^+$ T lymphocytes latently infected with HIV-1 that become $p24^+$ upon mitogen stimulation. In latently HIV-1-infected T cell lines (J-Lat 6.3/8.4/9.2, J1.1) that display basal NKG2DL expression, treatment with suberoylanilide hydroxamic acid (SAHA) or other LARs simultaneously reverts viral latency and up-regulates MICA, MICB, and ULBP2 to a variable extent, with a significantly higher ligand induction on cells harboring reactivated HIV-1. Moreover, combination of two LRAs with different mechanisms of action such as a histone deacetylase inhibitor and a PKC agonist resulted in more efficient HIV-1 reactivation in association with higher NKG2DLs up-modulation. Of note, SAHA exposure sensitizes J1.1 cells to NKG2D-mediated lysis by NK cells with a significantly stronger effect on $p24^+$ if compared to $p24^-$ cells. Finally, we found that IL-2 and IL-15 potently boosted NKG2D expression and cytotoxicity of NK cells against SAHA-reactivated $p24^+$ target cells.

DISCUSSION: Treatment with LRAs simultaneously induces expression of latent HIV-1 and cell-surface NKG2DLs, thus sensitizing T cells with reactivated virus to NKG2D-mediated killing by NK cells.

CONCLUSIONS: We propose that combining immunotherapy with cytokines that enhance NKG2D expression and cytotoxicity of NK cells with administration of LRAs up-modulating NKG2DLs, represents a promising approach towards HIV-1 eradication.

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(632) Tumor immunology

IMMUNE-RELATED URINARY MOLECULES AS A DIAGNOSTIC AND PROGNOSTIC BIOMARKERS IN PROSTATIC CANCER

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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 TGFβ 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.

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DISCUSSION: Taken together, these data suggest that Treg, but mainly Th17 CD4+ Tcells 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.

