Phagocytosis was superior in M2 (IL10) and M2 (IL4) activated MDM than in M1 MDM. Anti-TNF agents but not TCZ or RTX induced an increase of phagocytosis in M1 MDM.
Conclusions: Anti-TNF agents upregulate M2 alternative pro-resolving markers and downregulate M1 inflammatory markers in macrophages. Our results need to be extended by transcriptional analysis and evaluated in RA patients.
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Disclosure of Interest: Y. Degboe Grant/research support from: Société Française de Rhumatologie, B. Rauwel: None declared, M. Baron: None declared, J.-F. Boyer: None declared, A. Cantagrel: None declared, A. Constantin: None declared, J.-L. Davignon: None declared
DOI: 10.1136/annrheumdis-2017-eular.1249

SAT0007 GROUP 3 INNATE LYMPHOID CELLS NUMBERS IN PERIPHERAL BLOOD PREDICT USTEKINUMAB (STELARA) THERAPY RESPONSIVENESS IN PSORIATIC DISEASE CASES WITH SUBCLINICAL IMAGING ENTHESOPATHY

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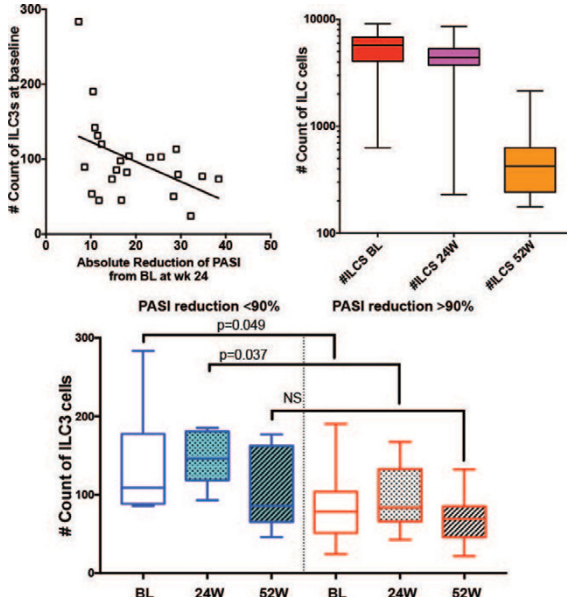
Background: Ustekinumab[1] targets the common p40 sub-unit of interleukin-12 (IL-12/interleukin-23 (IL-23). In patients treated with Ustekinumab for psoriasis where patients were selected on the basis of subclinical imaging enthesopathy, we have noted an improvement in subclinical imaging enthesopathy (Savage LJ et al submitted), raising the possibility that it may be possible to find a biomarker for predicting response to therapy in psoriatic disease. Innate lymphoid cells may be centrally involved in the pathogenesis of psoriatic skin and joints disease[2], since they express IL-23R receptor and are associated with IL-17/IL-22 production.
Objectives: This work was performed to test the hypothesis that peripheral blood ILC perturbations may be useful in defining response in psoriasis cases with imaging confirmed subclinical enthesopathy.
Methods: Peripheral blood collected at baseline (before therapy, 24weeks, 54 weeks) from patients in the MUSTEK trial (Ustekinumab in psoriasis cases who had ultrasound imaging confirmed subclinical enthesopathy) (n=23). Cellular immunophenotyping was performed density gradient separated PBMCs. Innate lymphoid cells were identified as lineage negative (CD3- TCR$\gamma\delta$- TCR$\alpha\beta$- CD19- CD14- CD11c- CD1a- CD303- FcεRI- CD34- CD123-) with positive expression of CD45, CD127. ILC2 cells were identified as Lineage- CD127+ and CRTH2 positive, while ILC3 were identified as Lineage- CD127+, CRTH2 – and CD117 (c-Kit) positive and further subdivided of NKp44+ and NKp44-. ILC1 were identified as lineage- CD127+ CD117-and CRTH2-. For data analysis we separated cases into PASI>90% or PASI <90% responders. The subclinical enthesopathy scores also fell significantly under therapy (Savage LJ data submitted)
Results: No correlation was found with total ILCs (ILC1,2, AND 3) (R=0.104, p=321, Spearman R) and therapy response. While, The absolute numbers of baseline ILC3s was inversely correlated in with the reduction in the PASI score (R -0.404, p=0308, Spearman R). The ILC3s also fell progressively under therapy. All the patients respond with reduction of PASI score mean 92.6% (range 65.8–100%), Interestingly, those patients with reduction below 90% of PASI score has a significantly higher absolute numbers of ILC3+ cells in peripheral blood at the baseline than PASI (n=6/23) than super-responder group (n=17/23)
Conclusions: Only peripheral blood ILC3s, but not other ILCs changes, correlate with the PASI score (disease activity), Furthermore, excellent responders (PASI reduction >90%) showed strong correlated with higher ILC3 population at the baseline. This may help to use ILC3 enumeration as predictive parameter for ustekinamab clinical therapeutic response and may be relevant to assessing novel biomarkers for subclincal arthropathy in psoriasis.



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Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.5347

SAT0008 DRUG THERAPY ENHANCES TOLEROGENIC PROPERTIES OF DENDRITIC CELLS IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: Dendritic cells (DCs) are known to contribute to the pathogenesis of rheumatoid arthritis (RA) through presentation of cartilage glycoprotein, production of proinflammatory cytokines and activation of Th1/Th17 responses. Along with stimulating activity, DCs may exhibit suppressive functions via capacity to induce T cell apoptosis/anergy and to generate regulatory T cells. Since these DCs have potential to control autoreactive T-lymphocytes, the enhancing of tolerogenic properties of DCs seems to be a new important strategy in treatment of RA. The experimental research in animals and human in vitro studies revealed the capacity of anti-rheumatic drugs to inhibit stimulating activity and to enhance tolerogenic functions of DCs. However, data concerning the in vivo influences of drug therapies on DC functions in RA patients are not available.
Objectives: The aim of our study is to investigate, whether drug therapies influence the properties of monocyte-derived DCs generated in the presence of IFNα (IFN-DCs) in RA patients, and if the effect of disease-modifying anti-rheumatic drugs differ from that of biological/pulse steroid therapy.
Methods: Thirty nine patients with RA with high and moderate activity of disease were recruited in this study. All patients fullfield ACR/EULAR criteria (2010). Nineteen patients received methotrexate, leflunomide, sulfasalazine or their combination (RA1). Twenty patients were at pulse therapy (methylprednisolone 500mg No. 3) or biological drugs (adalimumab or rituximab) (RA 2). All studies were performed after receiving informed consent. DCs were generated from blood monocytes culturing for 5 days with GM-CSF and IFN-α in the absence and presence dexamethasone, applied on third day. LPS as maturation stimuli was added on fourth day. The expression of CD14, CD83, CD 86, B7H1, HLA-DR, TLR-2 on the surface of DCs was measured by flow cytometry. The functions of DCs were evaluated by measuring cytokine production and DC allostimulatory activity in mixed lymphocyte culture.
Results: Both DC-RA1 and DC-RA2 where shown to display impaired maturation evidenced by elevated expression of CD14 and decreased number of mature (CD14-CD83+) DCs. Wherein, DCs-RA2 demonstrated several additional differences, including increased number of intermediate CD14+CD83+ cells (compared with donors DCs), higher expression of inhibitory molecule B7-H1 (PD-1L) (compare with donors DCs and DCs-RA1) and tendency to lower expression of CD86 and higher expression of TLR-2. Besides, DCs-RA2 produced higher concentrations of IL-6 and had 2-fold lower allostimulatory activity then DCs-RA1. These differences together with phenotypic changes suggested more pronounced tolerogenic properties of DCs-RA2. Despite the revealed DC differences in RA1 and RA2 patients both types of DCs preserved in vitro sensitivity to dexamethasone, that suppressed the production of TNF-α and reduced allostimulatory activity.
Conclusions: The data obtained indicate that, IFN-DCs from RA patients at drug treatments are characterized by tolerogenic properties, which are more pronounced in patients with biological or pulse steroid therapy.
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.2408

SAT0009 DISTRIBUTION AND INTRACELLULAR SETTITNG OF GRANULYSIN IN WOMEN WITH KNEE OSTEOARTHRITIS

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Background: The role of the cell-mediated immune response is recently recognized in osteoarthritis (OA) (1). Granulysin (GNLY) is mediator of cellular immunity and cytotoxic molecule expressed in T and NK cells in regulatory (15 kDa) and cytotoxic (9 kDa) forms (2). Cytotoxic 9 kDa form of GNLY mediates apoptosis of eukaryotic cells (3) and might be responsible for silent unscheduled apoptosis of joint tissue cells in patients with OA without clinically recognized

systematic immune reaction, as measured by erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) (4). We investigate GNLY distribution and intracellular setting in peripheral blood lymphocytes of patients with OA.

Objectives: Women with knee OA (17), and age and sex appropriated control (17) were tested. All of them signed informed consent before sampling of peripheral blood (PB).

Methods: Medical history, clinical examination, X-ray and routine laboratory testing (ESR, CRP) were used for diagnosing OA. Peripheral blood mononuclear cells (PBMC) were isolated by gradient density centrifugation and used for multiple, simultaneous intracellular [total GNLY, 9 kDa GNLY, 15 kDa GNLY and Lysosomal-associated membrane protein-1 (LAMP-1)] and surface antigens (CD3 and CD56) detection by immunofluorescence. Data were analyzed by flow cytometry or confocal microscopy.

Results: In OA and control samples the percentage of total GNLY+ cells and GNLY expressing NK and T cells did not significantly differ and correlate with ESR and CRP. The frequency of GNLY+ cells was always higher in natural killer (NK) then in T cells and 9 kDa GNLY dominated over 15 kDa GNLY. However, 9 kDa GNLY co-localized more with the marker of cell degranulation, LAMP-1 in polarized granules of OA patients when compared to the control or to the 15 kDa GNLY.

Conclusions: The increase in the expression of cytotoxic (9 kDa) over regulatory (15 kDa) GNLY form in PBMC and its intracellular setting in polarized exocytic granules suggests the involvement of activated GNLY$^+$ lymphocytes in the immunopathogenesis of knee OA, indispensable of ESR and CRP.

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Acknowledgements: The experiments were financed by the University of Rijeka (No.13.06.1.1.06) and "Thalassotherapia- Opatija", Opatija, Croatia.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.5499

SAT0010 THE DAMP PROTEIN S100A8/A9 IS CRUCIALLY INVOLVED IN MYELOID-DERIVED SUPPRESSOR CELL (MDSC) DIFFERENTIATION AND FUNCTION IN COLLAGEN-INDUCED ARTHRITIS

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Background: Over the last years, the differentiation and activation of MDSC under tumor conditions has been studied extensively. Various tumor-derived factors have been identified in promoting the accumulation of this suppressive cell population. Recent findings determined the two alarmins S100A8 and S100A9 as important factors in the differentiation of MDSC during tumor conditions. However, little is known about the S100A8/A9 driven expansion and activation, as well as the relevance of these cells in autoimmune diseases such as rheumatoid arthritis.

Objectives: We therefore analyzed the mechanisms involved in S100A8/A9 driven MDSC accumulation and their functional importance in a mouse model of rheumatoid arthritis.

Methods: To investigate the effect of S100A8/A9 on MDSC differentiation, bone marrow cells from wild type (wt), S100A9 knockout (A9ko) and S100A9 transgenic (A9tg) mice were analyzed. Accumulation of MDSC and their phenotypical characterization was performed by FACS analysis and functional characterization including arginase activity, NO- and ROS-production and T cell proliferation assays.

The role of S100A8/A9 and MDSC in arthritis was investigated using the collagen-induced arthritis (CIA) mouse model. Accumulation of MDSC in different organs was analyzed by FACS and systemic S100A8/A9 levels were measured by ELISA. Ex vivo functional analysis of purified MDSC was performed to assess the potential of these MDSC to inhibit T cell responses.

Results: Our in vitro studies reveal that, in the presence of S100A8, myeloid progenitor cells differentiate to immature cells that phenotypically as well as functionally resemble MDSC. These cells are characterized by co-expression and up-regulation of arginase activity, NO- and ROS-production, and exhibit strong suppressive effects on the proliferation of both CD4- and CD8-positive T cells. Furthermore, accumulation of MDSC by extracellular S100A8 was found to be mediated via the Toll-like receptor 4 signaling pathway. In addition, lack of intracellular S100A8/A9 results in a decreased number of MDSC as well as a reduced suppressive activity of these cells, implying a dual role of these proteins for MDSC differentiation and function.

Investigating the role of S100A8/A9 and MDSC in a mouse model of CIA, a clear correlation between disease scores, MDSC numbers and systemic S100A8/A9 levels was observed. Furthermore, disease activity was reduced in wt and A9tg mice compared to A9ko mice and was in line with an increased accumulation of MDSC in the lymph nodes. Next to an enhanced suppressive activity of MDSC isolated from lymph nodes of wt and A9tg mice, these MDSC promoted the accumulation of regulatory T cells (Treg) whilst suppressing the differentiation of TH17 cells. In contrast, MDSC isolated from lymph nodes of A9ko mice had no effect on Treg differentiation and did not inhibit TH17 emergence.

Conclusions: Our in vitro results clearly show a S100A8/A9 dependent accumulation of cells that phenotypically as well as functionally resemble MDSC also under non-tumor conditions. In vivo data strongly support the importance of these findings. By influencing MDSC accumulation and function, S100A8/A9 is critically involved in regulating the disease outcome in rheumatoid arthritis, implying an important role of S100A8/A9 derived MDSC in regulating immune reactions during autoimmune diseases.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.6683

SAT0011 THE ACTIVATION OF MUSCARINIC ACETYLCHOLINE RECEPTORS INFLUENCES THE ONTOGENY OF NEUTROPHILS

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Background: There is growing evidence that nervous and immune system communicate with each other through soluble mediators.[1] Immune cells such as neutrophils express muscarinic acetylcholine receptors (mAChR), which are neuroimmune receptors and highly prevalent in the nervous system.[2] Aberrant neutrophil functioning plays an important role in various autoimmune diseases. Dysregulation of neutrophil immune responses such as oxidative burst and migration is one of the key mechanisms leading to tissue damage in autoimmune diseases.[3] However, the impact of mAChR activation on neutrophils remains contradictory.

Objectives: We aimed to determine effects of muscarinic receptor activation on development and functions of neutrophils.

Methods: Neutrophils were isolated from peripheral blood of healthy donors by dextran sedimentation. After one hour in the absence or presence of the natural ligand acetylcholine (Ach) (10nM-100μM) or the muscarinic agonist oxotremorine-m (oxo-m) (10nM-100μM), neutrophil respiratory burst was analyzed by dihydrorhodamine (DHR) flow cytometry assay and migration assessed by transwell assay in response to N-formylmethionyl-leucyl-phenylalanine (fMLP). Cells that migrated were quantified by flow cytometry. To analyze the effects mAChR activation on the development of neutrophils, HL-60 cells were incubated in the presence of DMSO (1%), oxo-m (100μM) or DMSO plus oxo-m. After 6 days, cells were harvested and expression of maturation markers (CD15, CD63 and CD16) as well as mAChR (M1-M5) were measured by flow cytometry.

Results: We observed no effects of mAChR activation on the respiratory burst of neutrophils. However, both ACh and oxo-m inhibited neutrophil migration in a dose-dependent manner, but with peculiar differences. By increasing acetylcholine concentrations, we observed a reduction of neutrophil migration in a directly proportional manner. On the other hand, while the lowest dose (10nM) of oxo-m inhibited migration most effectively, the increase of oxo-m showed inversely proportional effects on neutrophil migration. Thus, we aimed to investigate, if the highest dose of oxo-m has a different effect on neutrophils ontogeny. In agreement with the results obtained with neutrophils, the incubation of HL-60 cells with the highest dose of oxo-m showed no effect on oxidative burst and migration and induced no changes in the expression of mAChRs (M1-M5), CD16 and CD63 in HL-60 cells. However, we observed that it resulted in significantly increased surface levels of the neutrophilic lineage marker CD15.

Conclusions: Our data indicate a differential activation of mAChR affecting different steps of neutrophil ontogeny. Considering this finding, abnormalities in the activation of muscarinic receptors as have been observed in autoimmune diseases might contribute to neutrophil dysfunction and need further investigation.

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Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.6573

SAT0012 S100A8/A9 INCREASES THE MOBILIZATION OF LY6C HIGH MONOCYTES TO THE SYNOVIUM DURING EXPERIMENTAL OSTEOARTHRITIS

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Background: It is increasingly recognized that part of the pathology in osteoarthri-