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FRI0424 EQUAL PRESENCE OF CIRCULATING MAIT CELLS IN AXIAL SPA PATIENTS WITH ONLY AXIAL INVOLVEMENT AND AGE- AND SEX-MATCHED HEALTHY CONTROLS

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Background: Previous studies indicated a potential role for mucosal-associated invariant T (MAIT) cells in the pathogenesis of ankylosing spondylitis (AS).^{1,2} Active peripheral arthritis and extra-articular manifestations may influence the presence of circulating MAIT cells in AS.

Objectives: To investigate circulating MAIT cells in a homogenous group of axial spondyloarthritis (SpA) patients with only axial involvement in comparison to age- and sex-matched healthy controls (HC). Secondly, to explore the association of MAIT cells with symptom duration and disease activity.

Methods: Consecutive axial SpA patients from the Groningen Leeuwarden axial SpA (GLAS) cohort without active peripheral arthritis, inflammatory bowel disease, psoriasis or uveitis were included. Patients with active infections or current use of biologics were excluded to rule out possible influence on the presence of circulating MAIT cells. Disease activity was assessed using ASDAS, BASDAI, and serum CRP levels.

The frequencies and absolute numbers of circulating MAIT cells were examined in peripheral blood of all studied samples by 5-color flow cytometry. Immediately after sampling, EDTA-blood was stained with anti-CD3, anti-CD8, anti-TCRV α 7.2, anti-CD161, and anti-TCRG δ . After staining, the cells were washed, fixed, and analyzed immediately on FACS. MAIT cells were identified phenotypically as CD3⁺CD8⁺TCRV α 7.2⁺CD161^{high} cells.

Results: Of the 41 included axial SpA patients, mean age was 46 \pm 16 years, 73% were male, mean symptom duration was 23 \pm 14 years, and 78% were HLA-B27 positive. Mean ASDAS was 2.6 \pm 1.0, mean BASDAI was 4.4 \pm 2.5, and median CRP was 3 (range 2–30). 70%, 54% and 37% of axial SpA patients had ASDAS \geq 2.1, BASDAI \geq 4 or CRP \geq 5, respectively. HC had exactly the same age and sex distribution.

Both the percentages and absolute numbers of circulating MAIT cells were comparable between axial SpA patients and HC (Figure 1). In axial SpA patients, absolute numbers of MAIT cells correlated negatively (ρ =−0.339) with symptom duration. No significant associations were found between MAIT cells and disease activity, except for a negative correlation (ρ =−0.332) between frequency of MAIT cells and BASDAI (Table 1). There were no significant differences in MAIT cells between axial SpA patients with and without active disease according to ASDAS, BASDAI or CRP.

Table 1. Association of percentages and absolute numbers of MAIT cells with symptom duration and assessments of disease activity in axial SpA patients (n=41), Spearman correlation coefficients

| | % CD8 ⁺ MAIT | nr CD8 ⁺ MAIT |
|------------------------|-------------------------|--------------------------|
| Symptom duration (yrs) | -0.248 | -0.339* |
| ASDAS _{CRP} | -0.221 | 0.053 |
| BASDAI (0–10) | -0.332* | -0.126 |
| CRP (mg/l) | -0.137 | 0.030 |

*P-value <0.05.

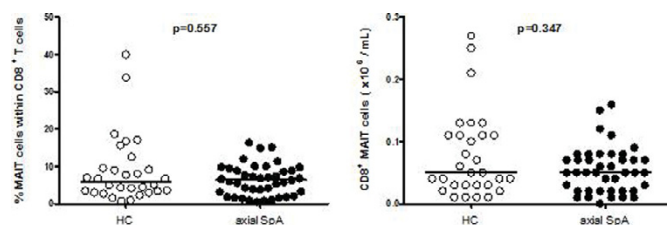


Figure 1. Percentages and absolute numbers of circulating CD8⁺ MAIT cells in axial SpA patients (n=41) and matched healthy controls (n=30), Mann-Whitney U tests.

Conclusions: In this homogeneous group of axial SpA patients with only axial disease, the presence of circulating MAIT cells did not differ from age- and sex-matched HC. No strong association was found between circulating MAIT cells and symptom duration or disease activity.

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FRI0425 ANALYSIS OF CELLULAR COMPOSITION AND CYTOKINE EXPRESSION IN THE SUBCHONDRAL BONE MARROW IN ANKYLOSING SPONDYLITIS

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Background: Ankylosing spondylitis (AS) is characterized by inflammation within the sacroiliac joints and at the spine including vertebral bodies and facet joints. In AS, bone destruction is followed by new bone formation leading to ankylosis of joints and syndesmophyte development. Histological studies of bone material from AS patients showed subchondral bone marrow changes, namely the transition of the bone marrow into granulation tissue which facilitates subchondral bone destruction but also promotes local bone formation [1, 2]. Currently, it is unclear what promotes the transition of the bone marrow into the granulation tissue.

Objectives: The aim of this study was to look for changes in the subchondral bone marrow in joints from AS patients that may precede and promote transformation into granulation tissue. Therefore, we analyzed the cellular composition of the bone marrow and determined local cytokine expression at subchondral regions of AS facet joints.

Methods: Facet joints were acquired from AS patients undergoing polysegmental correction surgery and compared to joints from autopsy controls. We performed immunohistochemical stainings to determine the number of T cells (CD3) and B cells (CD20), macrophages (CD68, CD163), monocytes (CD14), dendritic cells (DC; CD1a, DCsign) and myelopoietic cells (MPO). To correct for putative differences in fat cell content, software assisted image analysis was used to calculate the area of bone marrow covered by fat cells and the number of nucleated cells (DAPI) as well as myeloid cells. Cytokine expression was determined by mRNA in situ hybridization (TNF, IFN γ) or immunohistochemistry (TGF β , IL-10).

Results: We observed no difference in the number of adaptive immune cells, i.e. CD3⁺ T and CD20⁺ B cells, between AS and control joints and found no difference in T/B cell aggregates. Furthermore, no difference in the number of macrophages (CD163⁺, CD68⁺) and DCs (CD1a⁺, DCsign⁺) was found. The only difference in cellular composition appeared to be a slight trend towards a higher percentage of myelopoietic MPO⁺ cells/DAPI⁺ cells in AS joints compared to control joints. The overall number of DAPI⁺ cells as well as the fat content of the bone marrow was not different between AS and control joints.

Concerning cytokines, TNF mRNA expression was in general low but significantly increased (p <0.05) in AS versus control joints. No difference was found for IFN γ . The number of TGF β expressing cells was similar in AS and control joints while the number of IL-10 positive cells was decreased (p <0.01) in AS joints.

Conclusions: The results show a shift from pro-inflammatory to anti-inflammatory cytokine expression within subchondral bone marrow sites in AS joints while no major change in the cellular distribution of the major leukocyte subsets is found. The shift in cytokine milieu may contribute to transformation of the bone marrow into granulation tissue. It would be interesting to determine the cellular source of local cytokines, such as of IL-10.

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FRI0426 NO DEMONSTRABLE EFFECT OF IL-23 RECEPTOR VARIANTS ON CLINICAL MEASURES AND IL23/ IL-17 LEVELS IN ANKYLOSING SPONDYLITIS

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Background: IL23 binding to IL23 receptor (IL23R) is necessary for the maturation of Th17 cells and generation of proinflammatory IL17 and TNF in AS. IL23R variants have considerable impact on AS susceptibility in genome-wide association studies.

Objectives: To describe the effect of genotypic IL23R variants on proinflammatory cytokines and disease measures in AS.

Methods: Cross sectional cohort study of patients with established AS (n=334, 90% B27 +, mean age at study 45 years) included in a disease registry. IL23R genotyping for nonsynonymous SNPs (rs11209026 (protective allele A) and rs11209032 (risk allele A) was done by Taqman RT-PCR, while IL23, IL17, IL6 and TNF levels determined by sandwich ELISA and compared with age and gender matched healthy controls (n=72). Genotypic associations with clinical and serological features were analyzed with nonparametric methods.

Results: There was no significant difference between in AS patients and controls

regarding IL23 (276 vs 262 pg/ml, $p > 0.4$) and IL17 levels (184 vs 233 pg/ml, $p > 0.2$). Only 22 (6.6%) AS patients carried the protective rs11209032 A allele, while 206 (61.7%) carried the rs11209032 A risk allele ($p = 0.03$). There was no demonstrable influence of individual genotypes (A vs G, AA vs AG vs GG) or haplotypic combinations on BASFI, spinal function tests, CRP, ESR, IL-23 or IL-17 levels (all $p > 0.3$).

Conclusions: While there is a high prevalence of the IL-23R rs11209032 A risk allele in Caucasian AS patients, this has no demonstrable bearing on clinical disease measures or serum IL-23 and IL-17 levels.

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FRI0427 FIRST DESCRIPTION OF GAMMA DELTA T CELLS AT NORMAL HUMAN ENTHESES

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Background: Recent animal studies have suggested that $\gamma\delta$ T-cells accumulate at entheses, secrete IL-17 and are responsible for driving the spondyloarthritis (SpA) phenotype resulting from IL-23 overexpression in mice (1, 2). In humans examination of the immunological profile of entheses has been hampered by lack of tissue. Recently, we used a novel strategy to show that group 3 innate lymphoid cells are present at the human entheses (3). Here we extend our methodology to examine the broader immunological profile of human entheses and to determine if $\gamma\delta$ T-cells are also present.

Objectives: To characterise $\gamma\delta$ T-cells at human entheses and adjacent peri-enthesal bone

Methods: Human enthesal soft tissue (EST) and peri-enthesal bone (PEB) was harvested from normal spinous process in patients undergoing elective spinal orthopaedic procedures. Interspinous EST was dissected from PEB and enzymatically digested, followed by isolation of mononuclear cells. Flow cytometry was then used to determine the proportion of B-cells (CD45+, CD19+) NK cells (CD45+, CD3-, CD56+) and T-cells (CD45+, CD3+). T-cells were then sub divided based on expression of CD4 (T-helper cells), CD8 (Cytotoxic T-cells) and TCR $\gamma\delta$ ($\gamma\delta$ T-cells). All enthesal data was compared to age-matched peripheral blood from healthy controls.

Results: Enthesal digests contained on average a lower proportion of T-cells compared to peripheral blood ($p = 0.018$). However, the proportion of T-cells not expressing either CD4 or CD8 was greater in enthesal tissues ($p = 0.021$), this population was largely composed of $\gamma\delta$ T-cells. As a proportion of T-cells $\gamma\delta$ T-cells were 6 fold more numerous in EST compared to peripheral blood ($p = 0.024$), and PEB had 3 fold more. 37% of EST $\gamma\delta$ T-cells expressed CCR6 this compared to 26% and 34% in PEB and peripheral blood respectively.

Conclusions: $\gamma\delta$ T-cells are present in normal human entheses and $\gamma\delta$ T-cells constitute a greater proportion of the T-cell pool compared to peripheral blood, making it likely that they represent a tissue resident population. Additionally, we observed a very similar proportion $\gamma\delta$ T-cells that expressed CCR6, a functional marker for IL-17 production, as was observed in mice (2). This is the first description of $\gamma\delta$ T-cells at the human entheses and offers tentative confirmation of findings in mouse models where these cells play a key role in SpA pathogenesis.

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FRI0428 THE JAK1-SELECTIVE INHIBITOR, FILGOTINIB, INHIBITS INFLAMMATION PATHWAYS OBSERVED IN AN IL23-INDUCED PSORIATIC ARTHRITIS MOUSE MODEL

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Background: Psoriatic arthritis (PsA) is a heterogeneous chronic inflammatory disease characterized by musculoskeletal involvement and extra-skeletal manifestations such as psoriasis, uveitis and Inflammatory Bowel Disease (IBD). The importance of several pro-inflammatory cytokines, in addition to TNF α , IL-12

and IL-23 which are targets of current treatments, suggests that novel therapies may benefit patients. The JAKs (a family of 4 non-receptor tyrosine kinases) are crucial for the signaling of many pro-inflammatory cytokines. In this regard, the JAK1-selective inhibitor filgotinib (GLPG0634, GS-6034) has shown efficacy in patients with rheumatoid arthritis (RA), a disease that shares some hallmarks with PsA, as well as in Crohn's disease (CD), making this molecule a potential therapy for PsA.

Objectives: To gain insight into filgotinib mode of action using a PsA preclinical model by analysing the gene expression signature of filgotinib in mouse phalanges and colon tissues from an IL23-induced PsA mouse model.

Methods: Spondyloarthritis was induced by hydrodynamic injection of IL-23 enhanced Episomal Expression Vector (EEV). Animals were treated with filgotinib or vehicle from day 10 (therapeutic mode) and sacrificed after 16 days of treatment. RNAs were extracted from the phalanges and the proximal colon, and transcriptome assays were performed using the Agilent SurePrint G3 mouse chip. Data analysis was performed using empirical Bayes methods and linear models (limma BioConductor package).

Results: In mice, IL-23 induced changes in the transcriptome in both phalanges and colon that were marked by effects on genes related to the IL-23/T H 17 axis. Microarray analysis performed on mouse phalanges and colon revealed that filgotinib partially reversed the impact of IL-23 on gene expression in colon and in phalanges. In both tissues, filgotinib signature was different but some impacted biological programs were similar. A consistent interferon signature was counteracted by filgotinib in both tissues with decreased expression of common genes such as Apobec3, Gbp8, ligp1 and Oas3. Several markers of inflammation or associated with IL-23 activity were also decreased with common on Kynu and Cd96 gene expression in both tissues. Of interest, filgotinib repression on inflammatory gene expression was stronger in colon compared to phalanges (IL1b, Clec4e). Moreover expression of some genes involved in gut homeostasis that were induced by IL-23 were decreased by filgotinib in the colon, notably Fpr2 (receptor for formyl peptides) and Mmp7. In phalanges, gene expression associated with IL-23-induced disease was also reversed by filgotinib treatment. Il19, Mtlc1 and Tlr1 which are key mediators in psoriasis, or Rankl that is involved in bone remodeling in PsA were differently regulated by IL-23 and filgotinib.

Conclusions: Systemic expression of IL-23 in mice generated a PsA phenotype that was associated with altered gene expression in diseased tissues. A strong interferon signature was reversed by filgotinib as were several inflammation and disease markers. Together with the previous Phase 2 clinical results in RA and CD, these data support the study of filgotinib for the treatment of PsA patients.

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FRI0429 DYSREGULATION OF THE SPLICING MACHINERY IN LEUKOCYTES FROM ANKYLOSING SPONDYLITIS PATIENTS IS ASSOCIATED TO DISEASE PATHOGENESIS

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Background: Ankylosing spondylitis (AS) is a chronic inflammatory disease, of unknown aetiology, associated to the development of several comorbidities such as atherosclerosis. Splicing is a post-transcriptional process involved in the RNA maturation. Recent studies have revealed that a pathological dysregulation of the splicing machinery or spliceosome is associated to several human diseases. Yet, the spliceosome alterations have not been described in AS.

Objectives: 1) To analyze whether dysregulation of the spliceosome is present in AS. 2) To evaluate the association between the alteration of this process and the clinical, inflammatory, oxidative, and atherogenic profiles present in this pathology.

Methods: Fourteen AS patients and 14 healthy donors (HDs) were included in the study. Disease function and activity status were analyzed using the BASFI and BASDAI. The expression of selected components of the major-(n=12) and minor-spliceosome (n=4), and splicing factors (n=28) was evaluated in purified monocytes, lymphocytes and neutrophils from patients and HDs (n=14 each) by Fluidigm methodology. Oxidative stress, inflammation and atherogenesis were evaluated by flow cytometry and RT-PCR. Endothelial function was determined by the post occlusive hyperaemia test using Laser-Doppler.

Results: Compared to HDs, a significant dysregulation in the expression of relevant splicing factors and spliceosome components was found in all the leukocyte subtypes from AS patients, being neutrophils which displayed higher number of altered molecules. Interestingly, a specific altered profile of major- and minor-spliceosome members, and splicing factors was observed when compared lymphocytes (U4, U6, NOVA1, RMB17), monocytes (PRP8, SF3B TV2, CELF4, ESRP2, RBM3, SAM68 TV1, SRSF10, TIA1) and neutrophils (U11, U2, U2AF2, U11, CA 150, ESRP1, PSF, PTB, SRM160).