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SAT0350

A ROLE FOR IL-17A IN THE SUPPRESSION OF SPINAL ENTHESEAL MESENCHYMAL STEM CELL ADIPOGENESIS WHILST SIMULTANEOUSLY FACILITATING OSTEOGENESIS.

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Background: Fat formation in the bone adjacent to the enthesis is an important but poorly characterised intermediate stage in new bone formation that occurs in the spine in AS. We and others have previously reported that IL-17A can increase

mesenchymal stem cell (MSC) mediated osteogenesis in normal and AS spinal tissue (1, 2).

Objectives: Herein we investigate the impact of IL-17A & TNF on MSC adipogenesis from spinal enthesis tissue.

Methods: Samples from healthy spinous process and interspinous ligament (n=14, median age = 53) were separated into the peri-entheseal bone (PEB) and entheseal soft tissue (EST) & enzymatically digested. Minimally passaged (<p3) MSCs were cultured in a complete adipogenic media, with some cultures supplemented with either IL-17A (50ng/ml), TNF (1ng/ml) or IL-17A & TNF for 3 weeks. Adipogenesis was quantitatively assessed by Oil Red O staining at day 21. IL-17A's effect on adipogenesis was further investigated by RNA extractions at Day 0, 3, 5, 7, 15 & 21 with supporting Oil Red O staining. 48 adipogenic and IL-17A target genes were used to investigate adipogenic progression and IL-17A effects on it over 21 day adipogenic differentiation.

Results: EST MSCs have a significantly higher adipogenic potential than matched PEB MSCs (n=14, p<0.001). TNF and IL-17A both cause significant decreases (all p<0.01, n=5) in adipogenesis for both PEB and EST MSCs. EST MSCs produced lipid vesicles by day-3 post-induction, with significant inhibition by IL-17A (p<0.01, n=4) seen from day 15 onwards. IL-17A caused a significant decrease in overall Oil Red O staining, and it changed the morphology of lipid vesicles with a majority of cells consistent with immature pre-adipocytes. This was supported by gene expression data, which indicated significant decreases in transcripts encoding vesicle fusion proteins (CIDEA p<0.05, PLIN1 p<0.01). PLIN1 also aids protection against lipolysis (4). Transcripts associated with osteogenesis (CEBPβ (3)) and MSC stromal support (CXCL12) were significantly upregulated in adipogenically-induced cultures stimulated with IL-17A when compared to control adipogenic media. TNF & IL-17A combination demonstrated that IL-17A drove the vesicle morphology changes, with TNF alone not showing the same vesicle changes.

Conclusion: Given the inverse link between MSC mediated osteogenesis & adipogenesis, these findings reveal a role of IL-17A especially on EST MSCs. The rapid formation of adipocytes seen in EST MSCs may be relevant to MRI determined peri-entheseal bone "shiny corners" due to post inflammation fat accumulation. Elevated transcripts associated with pre-adipocytes & undifferentiated MSCs support the idea of plasticity between early osteogenesis & adipogenesis. Downregulation of transcripts for proteins associated with protection against lipolysis allows for the rationalising of the gradual loss of the shiny corners seen in AS preceding subsequent new bone formation.

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SAT0351

CHEMOKINE PATHWAYS ARE ENRICHED IN PSORIATIC ARTHRITIS (PSA) SKIN LESIONS WITH INCREASED EXPRESSION OF ATYPICAL CHEMOKINE RECEPTOR 2 (ACKR2)

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Background: Skin in people with psoriasis has been comprehensively studied; uninvolved skin has abnormal gene expression. Less is known specifically about skin in PsA, the assumption being that it is identical to psoriasis. Chemokines and ACKR2 are among the upregulated genes in uninvolved psoriasis compared

to healthy skin[1]. ACKR2 is a scavenging receptor of inflammatory CC chemokines and has been proposed as a regulator of cutaneous inflammation in psoriasis. It has not been studied in PsA.

Objectives: To compare the transcriptome of PsA lesional, PsA uninvolved and healthy control skin and evaluate ACKR2 expression in PsA.

Methods: Biopsies were taken from healthy control (HC) skin and paired lesional and uninvolved skin from patients with PsA. Libraries for bulk RNA sequencing were prepared from polyA selected RNA and sequenced on NovaSeq 6000. Sequencing data were analysed using Searchlight2. ACKR2 mRNA expression was validated by qPCR. RNAscope was used to localise ACKR2 expressing cells and sections were co-stained with podoplanin or stained in serial sections with CD45. Chemokine protein expression in skin was evaluated using Luminex technology.

Results: Nine HC and 9 paired skin samples from patients with PsA were sequenced. The PsA skin lesions (PsA L) formed a distinct population in the transcriptomic principal component analysis (PCA) plot while HC and PsA uninvolved skin (PsA U) were overlapping. Only 15 genes were differentially expressed between HC and PsA U and none coded for chemokines. There were however significantly upregulated chemokines and receptors in PsA L. Unexpectedly, ACKR2 was the 2nd most upregulated chemokine receptor in PsA L with unchanged expression in PsA U compared with HC (PsA L vs HC log2fold 3.38, p.adj=9.51E-41; PsA L vs PsA U log2fold 3.58, p.adj=3.24E-45; PsA U vs HC log2fold -0.2, p.adj=0.732).

The upregulation of ACKR2 in PsA L and unchanged expression in PsA U was confirmed by qPCR. RNAscope demonstrated strong expression of ACKR2 in the suprabasal layer of the epidermis in PsA L. In HC and PsA U, only occasional ACKR2 positive cells were seen in the epidermis. ACKR2 was expressed in lymphatic vessel walls but was not observed in CD45+ leukocytes.

Provisional skin chemokine protein expression data showed poor correlation between mRNA levels and protein expression for the ACKR2 ligands CCL2, CCL3, CCL7, CCL8, CCL11, CCL13 and CCL22 in HC and PsA U, with negative correlation between ACKR2 mRNA expression and CCL2, CCL8 and CCL11 protein expression. In PsA L, chemokine mRNA correlated with protein expression, but protein expression of chemokine ligands did not correlate with ACKR2 expression.

Conclusion: This data set shows expected upregulation of chemokines and their receptors in PsA L but relatively unchanged gene expression in PsA U, which contrasts to previous studies in psoriasis. Notably, this study demonstrates a strong upregulation of ACKR2 in keratinocytes in PsA L, with unchanged expression in PsA U. The RNA expression and preliminary protein data suggest that ACKR2 has little effect on the levels of its ligands in PsA skin lesions. However, this study may have missed local effects of ACKR2 in the epidermis.

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SAT0352

AN UNSUPERVISED ANALYSIS IDENTIFIES A SPECIFIC IMPACT OF BIOLOGICS ON T LYMPHOCYTE PHENOTYPES.

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Background: It is currently not known if TNF or IL-17A inhibitors have an impact on immune cell frequencies in axial Spondyloarthritis (AxSpA). This question is important to understand the impact of biologics on the immune system. Data from

clinical trials didn't show significant modification on immune cells and especially on lymphocytes. But regarding the risk of infections linked to these treatments lymphocyte cell subsets are certainly disturbed. Moreover, biologics could affect subsets of cells with an unusual phenotype.

Objectives: To identify the phenotype of cell subsets affected by biologics.

Methods: We used an "unsupervised approach" to analyze CD4+ T cells and CD8+ T cells subsets. Contrary to a "supervised approach", this strategy takes advantages of the fluorescence emitted by all of the surface markers used to characterize the cells at the same time. The objective was on the one hand to overcome statistical problems related to the number of patients and the repetition of the tests and on the other hand to increase the sensitivity of the analysis by identifying and analyzing new cell populations. The first step was to cluster the cells based on a selection of 12 T cell markers characteristic of the classical cell subsets and the stage of maturation to obtain cell clusters with a phenotype based on the combination of these 12 markers. Then, we were able to describe "a posteriori" the change of frequency of the clusters identified. The second step was to create a visualization of the cells affected to confirm their existence in a classical flow cytometry gate. With this pipeline, we analyzed CD4 and CD8 T cells isolated from a group of AxSpA patients (n=7) before and after 3 months of TNF therapy and a group of patients (n=6) before and after 4 months of IL-17A therapy.

Results: We observed that after biologics CD4 and CD8 T cells frequencies did not change but there was a redistribution of the different clusters analyzed. Specifically, we identified for CD4+ T cells after anti TNF treatment an increase of 2 clusters (CD4+CD27+CD45RA+Va7.2intCD161int and CD4+CD27-CD45RA-CCR6+CD161int) and a decrease of 3 clusters (CD4+CD27+CD45RA+CRTH2intCD161int, CD4+ CD27+CD45RA+CXCR3+, CD4+CD27+CD45RA+gdiint CD161int) and for CD8+ T cells a decrease of 1 cluster after treatment (CD8+ CD27+CD45RA+CD161+CXCR3+) and an increase of 1 cluster (CD8+ CD27+CD45RA+). The clusters affected by anti-IL-17A therapy were different. For CD4+ T cells, we identified a decrease of 2 clusters (CD4+CD27+CD45RA+CXCR3+ CD161+ and CD4+CD27+CD45RA-CXCR3+CCR6+CD161+) and an increase of 2 clusters (CD4+CD27+CD45RA+gdiintCD161+, CD4+CD27+ CRTH2intCCR6+) and for CD8+ T cells a decrease of 1 cluster (CD8+CD27+CD45RA+ CXCR3+CRTH2intCD161int) and an increase of 1 cluster (CD8+CD27+CD45RA+CXCR3intCD161+).

Conclusion: We identified 5 different clusters in CD4+ T cells affected by anti TNF and 4 by anti-IL-17A. We identified 2 clusters in CD8+ T cells affected by anti TNF and 2 by anti-IL-17A. The phenotypes of these clusters were unexpected and raised new questions about the effect of biologics in AxSpA. We were also able to create a visualization of these cells affected by biologics in a "classic gating view" which will help us to perform scRNAseq. With this unique approach, we show an impact of biologics on the frequency of very specific subset of CD4+ and CD8+ T cells in AxSpA

Figure 1.

Disclosure of Interests: None declared

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SAT0353

STAT3 PHOSPHORYLATION IS INVOLVED IN THE DEVELOPMENTS OF INFLAMMATORY ARTHRITIS, ENTHESTITIS, AND NEW BONE FORMATION IN ANKYLOSING SPONDYLITIS

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