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 cells 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.

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SAT0353

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

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Background: Ankylosing Spondylitis (AS) is a chronic inflammatory rheumatic disease, which is characterized by the enthesitis, peripheral arthritis, and chronic inflammation of the spine, leading to bony ankylosis. Signal transducer and activator of transcription (STAT) family proteins are latent cytoplasmic transcription factors that convey signals to the nucleus. It is activated by IL-6, IL-23, and IL-22 through JAK-mediated phosphorylation. Moreover, genetic studies implicate interleukin-23 (IL-23) receptor signal, including STAT3 in the development of AS. IL-17A has recently emerged as a potential target that regulates the extensive inflammation and abnormal bone formation observed in AS. It was reported that STAT3 is a regulatory factor that induces Th17 cell development from naive CD4 T cells.

Objectives: The aim of this study is to investigate whether the STAT3 phosphorylation (stat3-p) inhibitor has a therapeutic effect on inflammation and new bone formation in AS.

Methods: Eight weeks after curdlan injection, SKG mice were treated with stat3-p inhibitor or mock as a control. Clinical and histologic scores for arthritis and enthesitis were evaluated. Synovial fluid mononuclear cells (SFMC) samples were obtained from AS patients. Inflammatory cytokine producing cells were analyzed using flow cytometry. Bone tissue samples were obtained from the facet joints of patients with AS at surgery. Primary bone-derived cells (BdCs) were isolated and cultured. The osteogenic differentiation was assessed in vitro for 3 weeks using ALP activity, Alizarin red S (ARS), Type I collagen, von kossa, and hydroxyapatite stains. Statistical analysis was performed using Prism 5.0 Software. A $p < 0.05$ was considered statistically significant.

Results: The stat3-p inhibitor significantly suppressed peripheral arthritis and enthesitis in SKG mice (figure 1). Inflammatory infiltration around the tendon–bone insertion site and along the tendon, as well as bony involvement were all reduced in stat3-p inhibitor-treated mice compared to control mice. We found that the levels of IFN- γ , IL-17, TNF- α were higher in AS Synovial fluid. A significantly decreased frequencies of IFN- γ , IL-17, TNF- α producing cells in AS SFMC were shown after stat3-p inhibitor treatment ($P < 0.01$).

In vitro experiment of bone formation, the stat3-p inhibitor suppressed ALP activity. In addition, there were significant decrease in Alizarin red S (ARS), Type I

collagen, von kossa staining scores due to stat3-p inhibitor at a concentration of 5 μ M. Light intensity of hydroxyapatite staining was also decreased by stat3-p inhibitor in a dose dependent manner (figure 2). Intriguingly, the stat3-p inhibitor suppressed osteogenesis in both early phase and late phase in AS-BdCs, down-regulating osteoblast-involved genes.

Conclusion: The stat3-p inhibitor had beneficial effects on reducing inflammation and new bone formation in AS animal model. In addition, stat3-p inhibitor suppressed bone formation in vitro experiment. These findings suggest that the stat3-p inhibitor could be a potential therapeutic agent for AS.

Bone-derived cell (AS facet joint)

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SAT0354

TYPE 3 INNATE LYMPHOID CELLS ARE KEY DRIVERS OF PSORIATIC ARTHRITIS

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Background: Psoriasis (PsO) and psoriatic arthritis (PsA) are two types of chronic inflammatory diseases that share a similar cytokines profile. About 30% of PsO patients also develop a joint involvement, but the underlying mechanism is still unclear. Innate lymphoid cells (ILC) and specifically the type 3 ILCs (ILC3s) have raised increasing interest as possible player in the pathogenesis of both diseases, as they produce the pathological key cytokine IL-17A.

Objectives: We addressed the contribution of ILC3s to the pathogenesis of PsO and PsA in patients as well as murine *in vivo* models.

Methods: 130 patients satisfying the Classification Criteria for Psoriatic Arthritis (CASPAR), 40 patients with PsO and 35 healthy volunteers were enrolled in the study. Information regarding clinical features, laboratory parameters were collected and psoriasis area severity index (PASI), disease activity score 28 (DAS28), disease activity in psoriatic arthritis (DAPSA), minimal disease activity score (MDA) were calculated. Magnetic resonance imaging (MRI) and high-resolution peripheral CT (HR-pQCT) were taken and PsA MRI score (PsAMRIS) was assessed. Flow cytometric analysis was performed and IFN γ -producing ILC1s, IL-4/IL-5-producing ILC2s and IL-17/IL-22-producing ILC3s were identified