the MMP-3 levels induced by IL-1 β by 88% ($n=7$, $p<0.001$) at 5 μ M and by 31% at 1 μ M ($n=7$, ns). In direct comparison to tofacitinib and baricitinib the IL-1 β (20 ng/ml) induced IL-6 increase was only decreased by peficitinib and filgotinib but not by tofacitinib or baricitinib even at 10 μ M. Tofacitinib only blocked the specific IL-6 effect e.g. induced by the combination of the soluble IL-6 receptor (sIL-6R) and low doses of IL-1 β ($p<0.01$).

The proliferation of RASF was attenuated by tofacitinib, baricitinib and peficitinib at 5 μ M. Peficitinib exerted the strongest decrease of about 70% ($p<0.001$, $n=4$) and it was the only inhibitor attenuating the proliferation at 1 μ M (23%, $p<0.05$, $n=4$). Peficitinib also decreased the migration of RASF by 38% at 1 μ M and by 92% at 5 μ M. The observed effects were not mediated by apoptosis and even after 48h peficitinib did not act cytotoxic on RASF. In contrast to migration, short-term adhesion onto a plastic surface was not affected by peficitinib.

Conclusion: Peficitinib and filgotinib modulated the IL-1 β -induced response of RASF. Peficitinib also inhibited the proliferation and the migration of RASF. Therefore, especially peficitinib could be able to reduce the aggressive pannus formation in RA patients.

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THU0027

INHIBITION OF TH17 CELLS IN PSORIATIC ARTHRITIS THROUGH ACTIVATION OF THE ARYL HYDROCARBON RECEPTOR IS DEPENDENT UPON DISEASE ACTIVITY

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Background: Th17 cells have been implicated in the development of psoriatic arthritis (PsA) and psoriasis and are a major target for therapy. Th17 cells exhibit plasticity and can transdifferentiate to other effector cell types. Th17 cells that lose expression of IL-17 in favour of IFN- γ (non-classical Th1 cells) contribute to the pathogenesis of autoimmune diseases and are found in the joints of patients with inflammatory arthritis. Conversely, induction of IL-10 in Th17 cells has been shown to suppress their pathogenic potential. The Aryl Hydrocarbon Receptor (AhR) is a nuclear receptor with pleiotropic effects on immune function. The AhR ligand, 6-formylindolo[3,2-b]carbazole (FICZ) has been shown to induce or suppress Th17 cell differentiation and promote generation of tolerogenic FoxP3+ T regs or Type 1 regulatory cells (Tr1) that produce IL-10. FICZ also reduces psoriasis inflammation in a model of skin inflammation through AhR activation¹. FICZ could thus play a regulatory role to control inflammation in PsA.

Objectives: To evaluate the immunomodulatory effects of FICZ on IL-17, IL-10 and IFN- γ production from human CD4+ T cells in PsA and healthy controls (HCs)

Methods: Whole PBMCs and purified CD4+ T cells were isolated from HCs, untreated or DMARD treated (UNT/DMARD) PsA patients and anti-TNF α (adalimumab and etanercept) treated patients. Cells were stimulated with anti-CD3/CD28 (α CD3/28) (1 μ g/ml) and cultured for 3-5 days in the presence or absence of FICZ (250nM). To some cultures 2,3,7,8-Tetrachlorodibenzodioxin (TCDD) (10nM) was used as a control AhR ligand, and IL-21 (30ng/ml) as a positive control for IL-10 production. Cells were then re-stimulated with PMA/ionomycin and intracellular production of IL-17, IFN- γ and IL-10 measured by FACS.

Results: The percentage of IL-17+ CD4+ T cells was reduced in the peripheral blood of HCs and PsA patients after 3 days (healthy $p=0.0098$, UNT/DMARD $p=0.0004$, anti-TNF α $p=0.0012$) and 5 days (healthy $p=0.0005$, UNT/DMARD $p=0.0012$, anti-TNF α $p=0.0012$). The percentage of IFN- γ + CD4+ T cells was reduced in HCs and PsA patients (HC $p=0.016$, UNT/DMARD $p=0.001$, anti-TNF α $p=0.001$) after 3 days culture with FICZ but this effect was only sustained for HCs ($p=0.02$) and anti-TNF α treated patients ($p=0.01$) after 5 days of culture. No ligand specific difference in IL-17 suppression was seen between FICZ and TCDD. No difference in baseline IL-10 expression was seen between treatment groups. However, IL-10 production was significantly enhanced by FICZ in the anti-TNF α patients group ($p=0.02$). This effect was not seen with TCDD nor IL-21 alone. The addition of IL-21 to cultures from anti-TNF α patients treated with FICZ further enhanced IL-10 expression ($p<0.05$). Increase in IL-10 by FICZ was associated with a reduction in IL-17. Upon stratifying patients by swollen joint count (SJC) and Psoriasis Area Severity Index (PASI) into high (SJC $\geq$ 3, PASI $\geq$ 5) and low (SJC $<$ 3, PASI $<$ 5) disease activity, the percentage of IL-17+ CD4+ T cells after 3 days culture with FICZ was reduced for patients with low PASI score ($p=0.02$). A similar trend was seen for those with low SJC ($p=0.06$). In contrast, stimulation with FICZ did not regulate Th17 cells in patients with high PASI score or SJC.

Conclusion: The AhR ligand FICZ suppresses IL-17 production in CD4+ T cells of PsA patients and HCs and IFN- γ in HCs and anti-TNF α treated patients. FICZ promotes IL-10 production in CD4+ T cells from anti-TNF α treated patients and this effect is enhanced in the presence of IL-21. CD4+ T cells from patients with high disease activity are more resistant to FICZ mediated IL-17 suppression. Use of AhR ligands may thus be a useful therapeutic adjunct to conventional and biological therapy in PsA.

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THU0028

COMPARISON OF IL-17A AND TNF INDUCED CYTOKINE SECRETION BY RHEUMATOID AND PSORIATIC ARTHRITIS SYNOVIAL FIBROBLASTS AND THEIR INHIBITION BY BIOLOGICS

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Background: Rheumatoid arthritis (RA) and psoriatic arthritis (PsA), although similar in several respects, display clinical as well as therapeutic differences. This includes, for example, the higher effectiveness of the anti-IL-17A biologic secukinumab in PsA than in RA patients. Synovial fibroblasts (SF) are one of the key effector cell types in the pathophysiology of RA and PsA. We hypothesized that RASF and PsASF respond differentially to IL-17A and its biologic secukinumab and that this might contribute to the difference seen in the therapeutic response.

Objectives: To examine the effect of the two cytokines IL-17A and TNF- α on RASF in comparison to PsASF as well as the effect of their corresponding biologics. To analyze the effect of the IL-17A homolog IL-17F.

Methods: SF were isolated from synovium from PsA or RA patients undergoing surgery. SF from RA and PsA patients were stimulated with recombinant IL-17A, IL-17F and TNF- α alone or with respective combinations. Dose-response curve analysis was performed with IL-17A. The biologics secukinumab and adalimumab were used to block the effects on the SF. As a measure of the proinflammatory response, secretion of the cytokine IL-6 was quantified using an immunoassay.

Results: IL-6 secretion was induced by IL-17A in RASF as well as PsASF (IL-17A: 13.7-fold $\uparrow$ vs 6.9-fold $\uparrow$; $n=3$). Although sharing the same receptor, the IL-17A homolog IL-17F alone caused no induction of IL-6 secretion in SF. However, when combined with TNF- α , both IL-17 isoforms, IL-17A and IL-17F, increased IL-6 secretion due to a strong synergistic effect with TNF- α . RASF ($n=3$) responded more strongly than PsASF ($n=3$) to the combined stimulation (IL-17A: 544-fold $\uparrow$ vs 127-fold $\uparrow$, IL-17F: 54-fold $\uparrow$ vs 27-fold $\uparrow$). Adalimumab and secukinumab were

similarly effective in abolishing the synergistic effect of IL-17A + TNF- α in RASF and PsASF.

Conclusion: According to our data, the differences in the therapeutic effectiveness of the anti-IL17A biologic secukinumab cannot be attributed to differential SF responses since the response to IL-17A alone and IL-17A together with TNF- α is not stronger for PsASF than for RASF and since secukinumab was similarly effective for both SF types. Furthermore, in a proinflammatory milieu with increased TNF levels, both IL-17A and IL-17F can contribute to promoting inflammation in the pathophysiology of PsA and RA.

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THU0029 FAS LIGAND REGULATES THE GENE EXPRESSIONS OF VARIOUS KEY MOLECULES IN RHEUMATOID SYNOVIAL FIBROBLASTS

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Background: Fas ligand (FasL) is a member of tumor necrosis factor superfamily (TNFSF6) and reported to contribute to synovial hyperplasia of rheumatoid arthritis (RA). Apoptosis of RA synovial cells through Fas/FasL pathway was inhibited by pro-inflammatory cytokines present within the synovium [1]. We previously reported that decoy receptor 3 overexpressed in rheumatoid synovial fibroblasts (RA-FLS) stimulated by TNF α protects the cells from Fas-induced apoptosis [2].

Objectives: In this study, we investigated the gene expression profiles regulated by FasL in RA-FLS to reveal how FasL is involved in the pathogenesis of RA.

Methods: RA-FLS were obtained during total knee replacement surgery from patients with RA. Four individual lines of primary cultured RA-FLS were incubated either with 1000 ng/ml of recombinant human FasL protein or the same volume of phosphate buffered saline as unstimulated control in reduced serum medium for 12h. Gene expressions were detected by microarray assay (Human Genome U133 Plus 2.0, GeneChip[®] 3' Expression Array; Thermo Fisher Scientific).

Results: Microarray data analysis revealed that FasL up-regulated or down-regulated the expression of various genes in RA-FLS. The function of regulated genes included transcriptional activator activity, positive regulation of metabolic process, positive regulation of cellular metabolic process, positive regulation of macromolecule metabolic process, positive regulation of nitrogen compound metabolic process, regulation of phosphorylation, positive regulation of biological process, regulation of phosphate metabolic process, regulation of MAPK cascade, and regulation of multicellular organismal process. The most up-regulated 3 genes by FasL were dual specificity phosphatase 6 (DUSP6), epiregulin (EREG) and interleukin11 (IL-11). The most down-regulated 3 genes by FasL were angiopoietin-like 7 (ANGPTL7), protein inhibitor of activated STAT2 (PIAS2) and growth differentiation factor 5 (GDF5).

Conclusion: DUSP6 regulates CD4⁺ T-cell activation and differentiation by inhibiting the T-cell receptor-dependent extracellular signal-regulated kinases 1 and 2 activations [3]. EREG is increased in patients with RA and associated with the development of cytokine-induced arthritis [4]. IL-11 regulates the growth and development of hematopoietic stem cells and decreases the pro-inflammatory cytokines and nitric oxide productions [5]. ANGPTL7 is pro-angiogenic factor [6] and promotes pro-inflammatory responses through the P38 signaling pathway [7]. PIAS proteins inhibit the activated STAT and are involved in the pathogenesis of RA [8]. GDF5 is associated with joint destruction of patients with OA and RA [9]. FasL regulates the expression of various genes in RA-FLS. Therefore, FasL may affect the pathogenesis of RA by regulating gene expressions of these molecules in RA-FLS.

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THU0030 THE DIFFERENTIAL EFFECT OF TNF-A AND IL-6R BLOCKERS ON THE EXPRESSION OF IL-17 AND ACTIVATED CD4⁺CD25⁺ T CELLS IN PATIENTS WITH PSORIATIC ARTHRITIS

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Background: TNF- α blockers are effective drugs for the treatment of psoriatic arthritis (PsA) and rheumatoid arthritis (RA). On the other hand, while interleukin-6 receptor (IL-6R) blockers have emerged as effective drugs in the treatment of RA, their effect in PsA has been disappointing. We hypothesized that the differential effect of TNF- α and IL-6R blockers in PsA may be mediated by the effect of these drugs on IL-17 expression, a cytokine profoundly involved in PsA and by their influence on activated CD4⁺CD25⁺ T cells.

Objectives: To evaluate the differential effect of adalimumab (ADA) (representing TNF- α blockers) and tocilizumab (TCZ) (IL-6R blocker) on the expression level of IL-17 and on the frequency of activated CD4⁺CD25⁺ T cells.

Methods: Levels of IL-17 mRNA expression were measured following *in vitro* co-culture of peripheral blood mononuclear cells (PBMC) derived from PsA patients with, ADA, TCZ at 10ug/ml or with medium alone as control for 3 days. Next, RNA was extracted and real-time PCR for IL-17 mRNA expression was performed. In addition, after 5 days incubation with the biologic agents the frequency of activated CD4⁺CD25⁺ T cells were analyzed by flow cytometry.

Results: The differential effect of ADA and TCZ on IL-17 mRNA expression and modulation of activated CD4⁺CD25⁺ T cells in culture of PBMC derived from PsA patients is shown in Figure 1. We found that IL-17 mRNA expression in PsA patients derived PBMC (n=20) was down-regulated by ADA. This down grading was significant in comparison with TCZ and medium control respectively ($p<0.0001$, $p=0.0003$). On the other hand, TCZ significantly up-regulated the expression of IL-17 as compared to medium control ($p=0.05$) (Figure 1A). The frequency of activated CD4⁺CD25⁺ T cells was down-regulated by ADA as compared to medium and TCZ, respectively ($p=0.03$, $p=0.005$), whereas activated CD4⁺CD25⁺ T cells were up-regulated by TCZ (although not significantly) as compared to medium control (n=60) (Figure 1B).

Abstract THU0030 – Figure 1. ADA and TCZ differentially modulates IL-17 mRNA expression and activated CD4⁺CD25⁺ T cells in PBMC derived from PsA patients. PBMC were co-cultured in the presence of ADA, TCZ or medium alone as a control. (A) After 72 h incubation RNA was extracted and real-time PCR for quantification of IL-17 was performed. (n=20). (B) After 5 days incubation change in %CD4⁺CD25⁺ T cells was determined by flow cytometry (n=60), ns=not significant.

Conclusion: Our data highlight the differential effect of ADA and TCZ on IL-17 expression level and on frequency of activated CD4⁺CD25⁺ T cells in culture of peripheral immune cells derived from PsA patients. This data suggest a new mechanism of action of ADA and provide a possible explanation of the inefficacy of TCZ in PsA.

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