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Figure 1. Clutred human aortic adventitial fibroblasts (HAAF) in vitro. HAAF were polygonal or spindle-shaped under the microscope. (A) Appearance of HAAF at generation 5, 100x; (B) Appearance of HAAF at generation 5, 400x.

Abstract THU0024 Figure 1.

Figure 2. Effects of T-614 on TNF- α stimulated cell viability (SF) of HAAF. In the absence and in the presence of TNF- α , SF of HAAF significantly decreased in 50, 100, 250 μ g/ml T-614 treated groups, compared with the control group within each group alone, and there was significant difference in SF among T-614 treated groups within each group; however, no significant difference was found in SF neither within control groups nor within T-614 groups treated with the same concentration. Data are shown as mean $\pm$ SD of 3 independent experiments, each in triplicate. $P < 0.05$ was considered significant. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ versus 0 μ g/ml T-614 within each group alone.

Abstract THU0024 Figure 2.

Figure 3. Effects of T-614 on TNF- α stimulated IL-8 production of HAAF. Compared with the control group within each group, in the absence of TNF- α , IL-8 level in the 5 and in the 100 μ g/ml T-614 treated groups was significantly higher, but there was no significant difference between the 50 μ g/ml T-614 treated group and the control group, and there was no significant difference within T-614 treated groups which were not stimulated with TNF- α ; in the presence of TNF- α , and there was a significant difference within T-614 treated groups stimulated with TNF- α , but not for 50 vs. 100 μ g/ml T-614 treated group; additionally, compared with the T-614 treated group under the same concentration but without TNF- α stimulation, the IL-8 level in the control group and in the T-614 treated groups stimulated with TNF- α was significantly higher. Data are shown as mean $\pm$ SD of 3 independent experiments, each in triplicate. $P < 0.05$ was considered significant. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ between the compared groups.

Abstract THU0025 Figure 2.

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THU0025

ANALYSIS OF POTENTIAL INTERACTIONS BETWEEN TENOCYTES AND SYNOVIAL FIBROBLASTS AFTER STIMULATION WITH CYTOKINES EXPRESSED WITHIN THE SYNOVIO-ENTHESAL COMPLEX

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Background: Psoriatic arthritis (PsA) is frequently associated with enthesitis. It has been proposed that inflammatory processes at the synovio-entheseal complex are involved in the pathogenesis of inflammatory arthritides including especially PsA. Besides IL-1 β and TNF- α , IL-15, IL-23 and IFN- γ are cytokines expressed within the synovium, tendons and entheses and some of which are already used as therapeutic targets. While

synovial fibroblasts (SF) are known key effector cells of cartilage destruction in inflammatory arthritides such as RA, tenocytes are a major component of tendons and entheses and play a central role in tendon inflammation observed in PsA.

Objectives: To investigate whether PsASF and tenocytes show significant interactions while being stimulated with the above cytokines alone as well as in combination with the aim to find out whether these may contribute to the pathogenesis of PsA.

Methods: SF were isolated from patients with PsA undergoing joint surgery. Human tenocytes were acquired commercially and isolated from hamstring tendon tissue of patients undergoing hamstring tendon ACL reconstruction. PsASF and tenocytes were stimulated with IL-1 β , TNF- α , IFN- γ , IL-15 and IL-23 alone and in combination. Direct cell co-culture experiments were performed at a 1:1 ratio of both cell types in parallel to experiments with single cell type cultures. IL-6 levels were measured by ELISA to quantify the immunological activation of the cells.

Results: PsASF as well as tenocytes showed strong responses to IL-1 β (tenocytes $\uparrow$ 173-fold, n=3; PsASF $\uparrow$ 56-fold, n=3) and TNF- α (tenocytes $\uparrow$ 10-fold, n=3; PsASF $\uparrow$ 9-fold, n=3) stimulation regarding IL-6 secretion. IFN- γ alone had only minimal effects on both cell types but acted synergistically when applied together with IL-1 β (tenocytes $\uparrow$ 218-fold, n=3; PsASF $\uparrow$ 129-fold, n=3) and TNF- α (tenocytes $\uparrow$ 24-fold, n=3; PsASF $\uparrow$ 19-fold, n=3). IL-15 and IL-23 alone showed no effect but the data suggest a small antagonistic effect against IL-1 β (tenocytes IL-15 $\downarrow$ 21%/IL-23 $\downarrow$ 27%, n=3; PsASF IL-23 $\downarrow$ 19%, n=3) and TNF- α induced IL-6 secretion. Overall, PsASF and tenocytes showed similar responses in the single cell type stimulation experiments. Co-culturing PsASF and tenocytes did not reveal any synergistic or antagonistic interactions in regards to any of the cytokines used.

Conclusion: Our data suggest that tenocytes and PsASF do not interact in a way that would promote inflammation within the synovio-entheseal complex. Also, as far as the induction of IL-6 is concerned, PsASF and tenocytes are not major target cells of IL-15 and IL-23. IFN- γ , however, may be able to promote inflammation in combination with other cytokines in both cell types.

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THU0026

TARGETING SYNOVIAL FIBROBLASTS IN RHEUMATOID ARTHRITIS BY PEFICITINIB AND FILGOTINIB

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Background: The Janus kinase inhibitors (JAKi) peficitinib and filgotinib are currently examined in clinical trials for treatment of rheumatoid arthritis. Both inhibitors are well tolerated up to doses causing Cmax values higher than 1 μ M [1,2]. This is in contrast to the approved dosages of tofacitinib and baricitinib reaching Cmax values below 0.5 μ M [3,4]. However, it is not known if the higher concentrations of peficitinib or filgotinib offer a benefit in treatment of rheumatoid arthritis.

Objectives: The aim of the study compared the effect of different JAKi on inflammatory response and functional behavior of fibroblast-like synoviocytes from patients with RA (RASf).

Methods: Human RASf were isolated and pretreated with JAKi. After stimulation with IL-1 β and JAKi with/without soluble IL-6 receptor (sIL-6R) the levels of IL-6 and MMP-3 were measured in supernatants by ELISA. The effect of different JAKi on proliferation of RASf was determined by a BrdU-incorporation assay. The influence of peficitinib on migration of RASf towards a FCS gradient was examined. For short-term adhesion assays, cells were treated with JAKi, detached and seeded in culture plates. The plates were extensively shaken and adherent RASf quantified by counting crystal violet stained cells. Cell viability, cytotoxicity and apoptosis were measured using commercially available assays.

Results: The IL-1 β (10 ng/ml) dependant IL-6 release of RASf was decreased by peficitinib (62%, p<0.001) and by filgotinib (30%, p<0.05, n=7) at 5 μ M. Peficitinib also decreased the IL-6 release at 1 μ M (24%, n=7). In contrast to filgotinib, the JAK-inhibition with peficitinib reduced

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