entry into the inflamed synovium and is regarded as a switch from acute to chronic inflammation. The non-canonical nuclear factor kappaB (NF-kB) pathway, with its main regulator NF-kB inducing kinase (NIK), may play a central role in this process.

Objectives To determine the effect of non-canonical NF-kB signalling on pro-angiogenic gene expression in RASF and on the angiogenic potential of human umbilical vein endothelial cells (HUVEC).

Methods RASF were stimulated with lymphotoxin $\alpha_1\beta_2$ (LT) or LIGHT to activate non-canonical NF-kB signalling, and/or TNF to selectively activate the canonical NF-kB pathway. To effectively block the non-canonical pathway, a dominant negative NIK expressing adenovirus (Ad.NIKdn) was used. Changes in pro-angiogenic gene expression were measured by RT-PCR. Furthermore, to determine the effect on EC proliferation, RASF and HUVEC were co-cultured in the absence or presence of LT, LIGHT, TNF or VEGF EC were visualised through immunohistochemical staining of CD31, which was then semi-quantitatively scored.

Results Gene expression analysis of RASF revealed an increase in mRNA levels of VCAM-1 and IL-6 after stimulation with LT, LIGHT and TNF. Increased expression of IL-8 and MMP-3 was also observed in cells treated with both TNF and LT or LIGHT. These levels were attenuated in cells transduced with Ad.NIKdn prior to stimulation, indicating that the increased expression levels were at least in part non-canonical NF-kB dependent. CCL2 and bFGF were expressed continuously by RASF regardless of stimulation. In the co-culture, proliferation levels of EC increased under all stimulation conditions, with LIGHT inducing almost a 2-fold increase ($p < 0.05$), which was comparable to VEGF ($p < 0.05$).

Conclusions RASF contribute to synovial angiogenesis through the expression of adhesion molecules, cytokines, chemokines, matrix remodelling enzymes and growth factors. We demonstrate that the non-canonical NF-kB pathway plays an important role in this process by regulating pro-angiogenic genes and promoting EC proliferation. Further investigation of this pathway could lead to novel non-canonical NF-kB blocking therapeutics that inhibit angiogenesis in RA, thereby halting disease progression.

A9.13 TNF-INDUCED- PROTEIN TYROSINE PHOSPHATASE NONRECEPTOR TYPE 2 (PTPN2) AS A NEGATIVE REGULATOR OF INFLAMMATION IN RHEUMATOID ARTHRITIS

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Background and Objectives Protein Tyrosine Phosphatase Non-receptor Type 2 (PTPN2) is a protein tyrosine phosphatase that plays a role in the development of autoimmune diseases. PTPN2 function has not been studied in rheumatoid arthritis (RA), although single nucleotide polymorphisms within the gene have been described to be associated with RA in genome wide association studies. Considering the involvement of PTPN2 in the regulation of key inflammatory pathways, our aim was to analyse the expression and function of PTPN2 in RA synovial fibroblasts (RASF).

Materials and Methods The expression of PTPN2 was assessed in synovial tissue and fibroblasts (passage 4–10) from patients with RA and osteoarthritis (OA) using immunohistochemistry, real-time PCR (w/o tumour necrosis factor α (TNFα), IL1β, LPS and hypoxia) and Western blotting. PTPN2 was silenced with silencing RNA. Levels of IL-6 and IL-8 expression were measured by commercially available ELISA in cell culture supernatants after silencing PTPN2 in RASF w/o stimulation with tumour necrosis factor α (TNFα). Apoptosis of RASF was evaluated by AnnexinV staining using flow cytometry after stimulation with TNF-related apoptosis-inducing ligand (TRAIL, 20 ng/ml) for 24 hours.

Results In RA synovial tissue, compared with OA, we observed a stronger staining of PTPN2 in both the lining and the sublining layer by immunohistochemistry. On mRNA level we confirmed this overexpression in RA synovial tissue (2.0 fold, $n = 4–5$). In isolated RASF the constitutive mRNA level of PTPN2 was higher than in OASF (1.6 fold, $p < 0.01$, $n = 10–16$).

Levels of PTPN2 were further upregulated in RASF after stimulation with inflammatory cytokines such as TNFα (10 ng/ml, 24 hours, 3.1 fold, $p < 0.05$, $n = 4$), TNFα and IL-1β (1 ng/ml, 2.3 fold, $n = 5$), LPS (100 µg/ml, 24 hours, 1.9 fold, $n = 5$) and by 1% hypoxia (1.3 fold, $n = 3$). Accordingly, basal PTPN2 protein expression was 2.0 fold higher in RASF than in OASF ($n = 4$) and TNFα upregulated levels of PTPN2 (1.7 fold). PTPN2-deficient RASF produced 2.4 times more IL-6 than scrambled siRNA transfected cells (mean ± SD pg/ml 11412 ± 6313 versus 28133 ± 12734, $n = 3$). On the other hand, levels of IL-8 were not affected (35800 pg/ml versus 24330 pg/ml, $n = 3$). Furthermore, after silencing, 34% increase in TRAIL-induced apoptosis was detected in RASF ($n = 5$) compared to scrambled controls.

Conclusions Our findings indicate that PTPN2, known to be involved in the pathogenesis of several autoimmune diseases, could be an important negative regulator of inflammation in RASF.

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A9.14 REGULATORY T CELL ABNORMALITIES IN PATIENTS WITH SLE SUGGEST AN IL-2-BASED IMMUNOTHERAPY

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Background Phenotypic and quantitative abnormalities of regulatory T cells have been described in association with systemic lupus erythematosus (SLE). Further, an impaired production of IL-2, the essential cytokine for Treg homeostasis, has been described for T cells from SLE patients. Here, we aim to substantiate the link between IL-2 deficiency and Treg abnormalities in SLE and to provide the basis for an IL-2 based immunotherapy.

Methods Phenotype, frequency and homeostatic status of Foxp3+CD127lo Treg and conventional Foxp3- T cell (Tcon) subsets were analysed by multi-colour flow-cytometry of PBMCs from SLE patients and healthy donors ex vivo and after in vitro low-dose IL-2 treatment. Disease activity was determined according to the SLE activity index (SLEDAI). Two-tailed Mann-Whitney U test or 2-way ANOVA test were used for statistical analysis between patient or treatment groups, Spearman's rank coefficient was used to calculate correlations with disease activity.

Results The frequency of CD25+ cells among Treg was significantly reduced in SLE patients compared to HC. Analysis of Ki67 expression revealed that proliferation was significantly increased in Tcon from SLE patients, resulting in a reduced Treg to Tcon proliferation ratio in SLE patients. The proliferation ratio correlated positively with the frequency of CD25+ Treg and inversely with disease activity. Treatment of SLE PBMCs with low-dose IL-2 in vitro resulted in increased frequencies of CD25+ cells among Treg and increased CD25 expression levels on Treg. Treg, but not Tcon proliferation was significantly increased under low-dose IL-2 treatment compared to untreated controls.

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Discussion Similar to what has been observed in lupus-prone mice and IL-2$^{-/-}$ mice, Treg from SLE patients show the classical hallmarks of IL-2 deficiency with loss of CD25 expression and impaired homeostasis. Our in vitro results show that these Treg defects can be restored by low-dose IL-2 treatment, suggesting IL-2 as a novel therapeutic target for SLE.

A9.15 REMISSION AS THE MAIN THERAPEUTIC TARGET: COMPARATIVE EFFICACY OF FOUR TREATMENT REGIMENS IN EARLY RHEUMATOID ARTHRITIS (RA) PATIENTS (PTS)

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Background Early RA contains the "the window of opportunities" for achieving the best results of therapy including remissions. It is very important to determine the influence of different treatment regimens on the remission rate in patients (pts) with early RA.

Objectives To compare development of remissions in pts with early (<2 years duration) RA who were randomly assigned to receive four different strategies of DMARDs treatment.

Methods One hundred forty-one pts with RA of less than 2 years duration (122 women, mean age 51 years, mean disease duration 24 weeks, mean DAS28 5.9; 64% RF-positive, 59% ACCP-positive) were randomised to receive one of the following treatment regimens: methotrexate (MTX, up to 20 mg/week, 35 pts); MTX plus prednisolone (P) 10 mg daily (MTX-P, 34 pts); MTX-P plus methylprednisolone (MP) 1000 mg intravenously on the first day of treatment (MTX-P-MP, 35 pts); leflunomide 20 mg daily (LEF, 37 pts). Duration of treatment was one year. Efficacy of therapy was assessed by EULAR criteria. Control points were 3, 6 and 12 months from the beginning of therapy. The primary endpoint was the development of remission.

Results At baseline all groups were comparable in their demographic, clinical and radiographic characteristics. One hundred twenty-seven pts completed the study. By the 3d month in the MTX group only 3.1% of the patients reached clinical remission, while in the combination groups of MTX with GC (including MP intravenously) 21.3% and 28.6%, respectively. By the 6th month the same tendency continued: combination of MTX with GC showed the greatest frequency of remissions – 33.3% and 23.5%, respectively. In the MTX and LEF monotherapy groups the corresponding figures were 15.2% and 20.6%, respectively. By the 12th month the frequency of remissions was significantly higher in pts treated with the combination of MTX and GC, including high doses of MP (37.5% and 29.4%, respectively) than in the MTX and LEF monotherapy groups (11.4% and 16.2%, respectively).

Conclusions In pts with early RA combined treatment with MTX and GC led to the significantly higher remission rate as compared with MTX and LEF monotherapy.

A9.16 SYNOVIAL FIBROBLASTS FROM PATIENTS WITH RHEUMATOID ARTHRITIS DIFFERENTIATE INTO DISTINCT FIBROBLAST SUBSETS IN THE PRESENCE OF CARTILAGE

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Background Rheumatoid arthritis synovial fibroblasts (RASF) migrate to distant tissue sites and damage articular cartilage. Using novel markers of RASF subsets to identify lining and sub-lining layer RASF we investigated the ability of RASF to undergo self-assembly, transmigration and cartilage degradation in vivo.

Methods Healthy human cartilage was co-implanted subcutaneously into the flank of SCID mice together with RASF. On the contralateral flank, cartilage was implanted without cells. After 60 days, implants and blood were analysed. Human cells were detected using immunohistochemistry for species-specific antibodies. For in vitro studies, RASF were isolated from patients with established RA and healthy controls and the expression of cellular markers were defined.

Results RASF at the ipsilateral implant differentiated into distinct fibroblast subsets in the presence of cartilage. Cells proximal to cartilage expressed markers of a lining layer phenotype (GP38, FAP, VCAM-1 and Cadherin-11). These cells attached to, invaded and degraded cartilage. Cells more distal to cartilage expressed sub-lining layer phenotype markers including CD248 and CD90. Cells expressing CD248 and CD90 were never observed in the lining layer (proximal to cartilage) and never invaded cartilage. The development of this stromal architecture mirrored that observed in vivo in the inflamed synovial membrane. This stromal pattern of distinct lining layer and sub lining layer differentiation was recapitulated in the contralateral implant that contained only cartilage. In addition, we demonstrate that RASF in vitro can be directed towards either a lining layer (GP38, FAP, VCAM-1 and Cadherin-11) or sub-lining layer phenotype (CD248 and CD90) following cytokine treatment. The lining layer, but not sub lining cell phenotype is associated with increased cartilage degradation in vitro.

Conclusions RASF have an activated cell phenotype ex vivo. In vitro and in vivo they display plasticity with the capacity to differentiate into distinct cell sub-populations that morphologically distinguish between the lining and sub-lining layer of the human joint. In vivo cell sub-population differentiation occurs locally at the site of engraftment and recapitulates the lining anatomy observed at the site of origin. This phenomenon is dependent on the release of tissue factors found present in the joint microenvironment following cartilage damage. This inflammatory microenvironment is required for cartilage destruction by RASF. Cellular therapies targeting RASF specific subsets are an unexplored but important therapeutic target to modulate inflammation and may provide an avenue to minimise joint damage in patients with RA.

A9.17 TARGETING THE EXPRESSION OF MIR-146A IN MOUSE INFLAMMATORY Ly6C^{high} MONOCYTE SUBSET FOR THERAPEUTIC INTERVENTION IN ARTHRITIS

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Background and Objectives Monocytes can give rise to different cell types including osteoclasts (OC), which play an important role in maintaining bone homeostasis by resorbing bone matrix. Circulating monocytes consist of at least two main functional subsets of immune cells, Ly6C^{high} and Ly6C^{low} monocytes, arising from a common progenitor in the bone marrow. Excessive and prolonged activation of inflammatory LyC6^{high} monocytes is a hallmark of many inflammatory diseases including arthritis. Among key molecular rheostats, micro (mi) RNAs are a class of regulatory RNAs that control basic biological functions and orchestrate inflammatory responses. Among master miRNAs of innate immunity, miR-146a exerts a negative retro-control on inflammation transduction signals and inhibits osteoclastogenesis. Despite aberrant increased expression in rheumatoid arthritis (RA), miR-146a is unable to properly down regulate inflammation, leading to prolonged TNF

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