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

production and increased OC, two arthritis hallmarks. Here, we investigated whether miR-146a was differentially regulated in both Ly6C^{high} and Ly6C^{low} monocyte subsets in healthy and arthritic conditions. Moreover, we developed techniques to deliver siRNA to LyC6^{high} monocytes in vivo and thus determined the specific impact of miR-146a overexpression in this particular monocyte subset on inflammation and osteoclastogenesis, in the context of arthritis.

Materials and Methods Subset monocytes were isolated from peripheral blood of arthritic and healthy mice by FACS sorting following membrane stainings. Transcriptomic analyses for miRNA expression were performed (n = 6) and differential miRNA expression levels were validated on individual samples using multiplex RT-qPCR (n = 13). Collagen-induced arthritic (CIA) mice were injected intravenously at disease onset with lipoplex containing either control or miR-146a mimic (0.5 mg/kg). Arthritis severity was monitored and OC differentiation was performed by stimulating bone marrow-derived monocytes (BMDM) with M-CSF and RANK-L miR-146a expression levels measured during OC differentiation. TRAP activity, OC numbers and nuclei per OC were quantified.

Results Transcriptomic analyses showed higher expression of miR-146a in Ly6C^{low} monocytes when compared to Ly6C^{high} monocytes, in both healthy and arthritis mice. In arthritis mice, expression of miR-146a in Ly6C^{high} monocytes is down-regulated as compared to healthy controls. During differentiation of BMDM into OC, miR-146a expression levels are down-regulated on day 2. Enforced expression of miR-146a in Ly6C^{high} monocytes upon intravenous injection of miR-146a-containing lipoplex leads to decreased bone erosion in mouse CIA. This effect was associated with a decreased number of mature OC and TRAP activity, as well as a reduced number of nuclei per OC.

Conclusions Overall, our results show that specific over-expression of miR-146a in Ly6C^{high} monocytes alters OC differentiation and decreased bone erosion in mouse CIA. These data also suggest that Ly6C^{high} monocytes might be the monocyte subset precursor of OC, and that targeting this specific monocyte subset might represent a therapeutic option in the context of arthritis to inhibit bone loss.

A9.18 POTENTIAL THERAPEUTIC APPLICATION OF HUMAN UMBILICAL CORD WHARTON JELLY DERIVED MESENCHYMAL STEM CELLS IN PRIMARY SJÖGREN'S SYNDROME

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Background and Objectives hUCMS are adult stem cells easy to retrieve in bulk, under acceptable ethical conditions. Their immune-modulatory and pro-differentiation properties have been widely demonstrated. Modulation of the immune system is mediated by both cell contact and soluble factors such as interferon (IFN)-γ produced by hUCMS. Very few evidence is currently available with respect to potential therapeutic application of hUCMS in systemic autoimmune disorders. In particular, no data have been published in primary Sjögren's syndrome (pSS) to date. Furthermore, we have recently developed an endotoxin-free alginate matrix which can be used to microencapsulate (CpS) different cell types and transfer them into a non-immunosuppressed host. These microcapsules containing pancreatic islets, upon approval by the Italian Ministry of Health for in vivo use, have been grafted into a cohort of patients with type 1 diabetes mellitus with no adverse effects, while proving to be immunoprotective. On this background, we aimed to assess the in vitro immune system modulation by IFN-γ pretreated CpS-hUCMS on T cells from pSS, with special regard to Th17- and Treg-cell subsets.

Materials and Methods Ten pSS patients and 5 healthy donors (HD) were enrolled. Peripheral blood mononuclear cells (PBMC) were obtained by density gradient from heparinised venous blood. Co-cultures of CpS-hUCMS and PBMCs were arranged at different ratios. Lymphocyte proliferation was assessed by CFSE dilution assay. Phenotypic analysis by flow cytometry for regulatory and effector T cell subpopulations was performed after culture. Real time PCR analysis and the evaluation of culture supernatants for cytokine expression are currently ongoing.

Results CpS-hUCMS were able to inhibit HD and pSS PBMC cell proliferation and this effect was inversely correlated to hUCMS number. Phenotypic analysis revealed a predominant Th17-cell response in SS which was promptly hampered by CpS-hUCMS Moreover, Th1-cell proliferation was fair and reduced Cps-hUCMS FoxP3 and IL-17 expression among CD4$^+$ T cells was also modulated by CpS-hUCMS, thereby suggesting that a Treg/Th17 rebalance.

Conclusions This is the first study evaluating the effects of hUCMSS on T cells in pSS. It appeared that CpS-hUCMS rebalance Treg/Th17 ratio and therefore may exert therapeutic effects in such disease. Furthermore, it is the first study that employs a new technology of drug delivery which may be applied in vivo in pSS patients.

10. Others

A10.1 ANALYSIS OF THE MIGRATORY POTENTIAL OF DERMAL FIBROBLASTS OF PATIENTS WITH SYSTEMIC SCLEROSIS

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Background Systemic sclerosis (SSc) is characterised by fibroblast-mediated progressive skin fibrosis. Subsequent involvement of internal organs leads to severe impairments of their function and lung fibrosis represents the most common cause of death in SSc. Molecular mechanisms of the spreading of SSc are largely unknown. Recent results showed the migratory potential of rheumatoid arthritis (RA) synovial fibroblasts (SFs). Based on these results, the aim of this study was the analysis of migratory and adhesive properties of SSc dermal fibroblasts (DFs) and their role in SSc-spreading with focus on organ involvement.

Methods SScDFs (in part GFP-transfected) were injected intracutaneously into SCID mice. After 14–25 days, parts of the skin, internal organs and blood were analysed. Besides fluorescence analysis, immunohisto- and -cytochemistry was performed using species-specific antibodies to detect human cells. To avoid cross-reactivity of the antibodies, additional real time PCR analysis with primers for human β2-microglobulin was performed after RNA isolation out of the respective organs and subsequent reverse transcription.

To analyse and to compare the adhesive behaviour of SScDFs with fibroblasts from other diseases and origins, multi-well culture plates were coated with Matrigel® (MG), growth factor-reduced (GFR) MG, or remained untreated. Cellular adhesion of SScDFs (n = 5), RASFs (n = 5), RADFs (n = 5), SFs (n = 2) and DFs of healthy individuals (n = 4) was analysed.

Results SScDFs or human cDNA, respectively, were not detected in any internal organ by fluorescence analysis, immunohistochemistry or real time PCR. Human SScDFs and human cDNA were only detectable in the murine skin at the injection site.

Fibroblasts of SSc patients, healthy SFs and DFs showed an increased adhesion to GFR MG compared to MG (SSc: GFR MG: 8.5 fold, MG: 8.2 fold; healthy SFs: GFR MG: 6.6 fold, MG: 4 fold; healthy DFs: GFR MG: 10 fold, MG: 7.6 fold). In contrast a reduction of cellular adhesion of RASFs (GFR MG: 4 fold, MG: 5.4 fold)