subsets were defined by flow cytometry. Healthy donors and patients with rheumatoid arthritis served as controls.

Results: We observed that ATA- and ACA-positive SSc patients harbour circulating B cells that secrete either ATA-IgG or ACA-IgG upon stimulation, depending on their serotype. In addition, we noted spontaneous secretion of ATA-IgG and, more remarkably, extensive secretion of ATA-IgA in ATA-positive patients. This degree of spontaneous, antigen-specific IgA secretion was specific for the ATA response in ATA-positive patients, while spontaneous ACA-IgA secretion was undetectable in the ACA-positive patient group. FACS experiments showed that spontaneously ATA-IgA secreting B cells were primarily present in the plasmablast compartment.

Conclusion: Our findings demonstrate that ATA-positive SSc patients harbour an activated ATA-IgG and ATA-IgA B cell response, as indicated by the spontaneous secretion of both ATA isotypes by circulating plasmablasts. Remarkably, the ACA B cell response was far less active and lacked the active IgA component which suggests a difference in the triggers driving these autoreactive B cell responses in patients. Moreover, the remarkable ATA-IgA secretion points towards a potential mucosal origin of the ATA response.

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SAT0298 IS INTERLEUKIN 6 A FACTOR OF FIBROGENESIS IN DERMAL FIBROBLASTS?

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Background: Interleukin 6 (IL-6) is known to have both pro- and anti-inflammatory properties, depending on the receptor activation. The classical IL-6 signaling via the membrane bound receptor is mainly anti-inflammatory, whereas signaling through the soluble receptor (sIL-6R) is pro-inflammatory/pro-fibrotic. However, the direct fibrotic effect of IL-6 stimulation on dermal fibroblasts is unknown.

Objectives: We investigated the fibrotic effect of IL-6 + sIL-6R in a dermal fibroblast model and assessed fibrosis by neo-epitope biomarkers of extracellular matrix proteins.

Methods: Primary healthy human dermal fibroblasts were grown for up to 17 days in DMEM medium with 0.4% fetal calf serum, ficoll (to produce a crowded environment) and ascorbic acid. IL-6 [1-90 nM]+sIL-6R [0.1-9 nM] alone or in combination with TGFβ [1 nM] were tested in three different donors. TGFβ [1 nM], PDGF-AB [3 nM] and non-stimulated cells (w/o) were used as controls. Tocilizumab (TCZ) with TGFβ + IL-6 + sIL-6R stimulation was tested in one donor. Collagen type I, III and VI formation (PRO-C1, PRO-C3 and PRO-C6) and fibronectin (FBN-C) were evaluated by validated ELISAs (Nordic Bioscience). Western blot analysis investigated signal cascades. Gene expression of selected ECM proteins was analyzed. Statistical analyses included One-way and 2-way ANOVA and area under the curve analysis.

Results: formation by the end of the culture period. The fibronectin and collagen type VI signal were consistent between the three tested donors, whereas the formation of type III collagen was only increased in one donor, but in several trials. Type I collagen formation was unchanged by IL-6 + sIL-6R stimulation. The gene expression of type I collagen was induced by IL-6 + sIL-6R. Western blot analysis validated trans-signaling by the IL-6+sIL-6R stimulation as expected.

IL-6 + sIL-6R stimulation in combination with TGFβ decreased fibronectin levels compared to TGFβ alone but did not reach the level of unstimulated fibroblasts. The formation of collagen type IV was generally unchanged with IL-6 + sIL-6R + TGFβ compared to TGFβ alone. Collagen type I and III formation was more scattered in the signals when IL-6 + sIL-6R was in combination with TGFβ, as the biomarker level could be either decreased or increased compared to TGFβ alone. In two studies the type I collagen level was synergistic increased by IL-6 + sIL-6R + TGFβ, whereas another study found the level to be decreased compared to TGFβ alone. The gene expression of fibronectin and type I collagen was increased with TGFβ + IL-6+sIL-6R compared to TGFβ alone.

Inhibition of IL-6R by TCZ in combination with IL-6 + sIL-6R did only decrease the fibronectin level with the lowest TCZ concentration (p=0.03). TCZ alone

decreased the fibronectin level in a dose-dependent manner (One-way ANOVA p=0.0002).

Conclusion: We investigated the fibrotic response of dermal fibroblasts to IL-6 + sIL-6R stimulation. IL-6 modulated the fibronectin level and modulated the collagen type III formation level in a somewhat dose-dependent manner. In combination with TGFβ, IL-6 decreased collagen type I and IV formation and fibronectin. However, in this study inhibition of IL-6R by TCZ did not change the fibrotic response of the dermal fibroblasts. This study indicated that IL-6 did not induce collagen formation in dermal fibroblasts, except type III collagen formation with high IL-6 concentration.

Figure:

Disclosure of Interests: Anne Sofie Siebuhr Employee of: Nordic Bioscience, Pernille Juhl Employee of: Nordic Bioscience, Morten Karsdal Shareholder of: Nordic Bioscience A/S., Employee of: Full time employee at Nordic Bioscience A/S., Anne-Christine Bay-Jensen Shareholder of: Nordic Bioscience A/S, Employee of: Full time employee at Nordic Bioscience A/S.

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SAT0299 PROLIFERATION, MIGRATION AND CONTRACTION ARE DIFFERENT BETWEEN TGFβ AND PDGF STIMULATED DERMAL FIBROBLASTS

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Background: Systemic sclerosis has vascular, inflammatory and fibrotic components, which may be associated with different growth factors and cytokines. Platelet derived growth factor (PDGF) is associated with the vasculature, whereas tumor necrosis factor beta (TGFβ) is associated with inflammation and fibrosis. We have developed a fibroblast model system of dermal fibrosis for anti-fibrotic drugs testing, but the effect of the fibroblasts mechanistic properties are unknown.

Objectives: We investigated different mechanical capacities of PDGF and TGFβ treated human healthy dermal fibroblasts in the SiaJ setting.

Methods: Primary human healthy dermal fibroblasts were grown in DMEM medium containing 0.4% fetal calf serum, ficoll (to produce a crowded environment) and ascorbic acid for up to 17 days. A wound was induced by scratching the cells at 0, 1, 3 or 7 days after treatment initiation. Wound closure was followed for 3 days. Contraction capacity was tested by creating gels of human fibroblasts produced collagens containing dermal fibroblasts and contraction was assessed at day 2 by calculating the percentage of gel size to total well size. Collagen type I, III and VI formation (PRO-C1, PRO-C3 and PRO-C6) and fibronectin (FBN-C)

were evaluated by validated ELISAs (Nordic Bioscience). Gene expression was analyzed after 2 days in culture. Statistical analyses included One-way ANOVA and student's t-test.

Results: Generally, PDGF closed the wound in half the time of w/o and TGF β , when treatment and cells are added concurrently or scratched one day after treatment initiation. When treatments were added 3 or 7 days prior to scratch, the cells treated with PDGF had proliferated to a higher degree than w/o and TGF β . A consequence of this, was that when cells were scratched the sheet of cells produced was lifted from the bottom and fold over itself, leaving a much greater scratch than in the other treatments. However, despite this increased gap the PDGF treated cells closed the wound at the same time as w/o and TGF β , confirming the results of the cells scratched at day 0 and 1.

Inhibition of contraction by ML-7 of otherwise untreated cells inhibited contraction significantly compared to untreated cells alone ($p=0.0009$). Contraction was increased in both TGF β and PDGF treated cells compared to untreated cells (both $p<0.0001$). TGF β + ML-7 inhibited the contraction to the level of w/o ($p=0.0024$), which was only 35% of ML-7 alone. In the contraction study the cells were terminated after 2 days of culture, thus prior to when biomarker of ECM remodeling is usually assessed. However, FBN-C was detectable and a significant release of fibronectin by TGF β and PDGF compared to w/o was found in the supernatant (both $p<0.0001$). The gene expression of FBN was only increased with TGF β ($p<0.05$) and not PDGF. ML-7 alone tended to decrease FBN-C and in combination with TGF β the FBN level was significantly decreased compared to TGF β alone ($p<0.0001$). However, the level of TGF β +ML-7 was significantly higher than ML-7 alone ($p=0.038$).

TGF β increased the gene expression of most genes assessed, except Col6a1. PDGF increased the gene expression of Col3a1, Col5a1 and Col6a1 above the critical fold change of 2, but only significantly in Col5a1 and Col6a1 (both $p<0.05$).

Conclusion: This study demonstrates that TGF β and PDGF have different mechanical capacities in human healthy dermal fibroblasts; TGF β increased gene expression of ECM related genes, such as collagens and have increased FBN release in the supernatant already 2 days after initial treatment. PDGF has increased contraction, proliferation and migratory capacities and less expression of ECM related genes and proteins.

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SAT0300

SERUM FROM "EARLY" SYSTEMIC SCLEROSIS PATIENTS ALREADY INDUCES THE ALTERNATIVELY ACTIVATED MACROPHAGE PHENOTYPE (M2) IN CULTURED HUMAN MONOCYTES

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Background: Alternatively activated (M2) macrophages seem to play a role in the fibrotic process of systemic sclerosis (SSc) as potential inducers of tissue fibrosis through their secretion of specific cytokines and chemokines, such as interleukin-10 (IL-10), macrophage derived chemokine (CCL-22) and pro-fibrotic metalloproteases (i.e. MMP9) (1-3).

Objectives: To investigate the presence of circulating cells belonging to the monocyte lineage showing an M2 phenotype in SSc patients (pts) and possible correlation with the clinical parameters of the disease. Moreover, to investigate if the treatment of cultured monocytes isolated from healthy subjects with serum derived from early SSc pts may induce their *in vitro* polarization into M2 macrophages.

Methods: Fifty female SSc pts (mean age 64 ± 13 yrs), fulfilling the EULAR/ACR criteria, and 27 gender-matched healthy subjects (HSs, mean age 57 ± 7 yrs) were considered at the Rheumatology Division of Genoa University after written informed consent. Nailfold videocapillaroscopy (NVC), serum SSc-related antibodies and skin involvement were investigated. Circulating cells belonging to the monocyte populations (CD45⁺ and CD14⁺ cells) were characterised by flow cytometry using specific surface markers of M2 phenotypes (CD204, CD206, CD163). Each SSc pt had been under stable treatment regimen for at least six months. Cultured monocytes, isolated by negative selection from peripheral blood mononuclear cells (PBMCs) of 8 HSs, stimulated for 48 hrs with 10% of serum of lSSc pts with "Early" NVC pattern, as well as serum of dcSSc pts with "Active" and "Late" NVC patterns. Cultured monocyte human cell line (THP1) was differentiated into macrophages (5ng/ml of phorbol myristate acetate) and then stimulated with SSc sera. The expression of CD204, CD206 (M2 markers) and CD68 was investigated by immunocytochemistry, whereas MMP9 secretion was investigated by zymography. Statistical analysis was performed using Mann-Whitney and Kruskal-Wallis tests, and correlations were explored by bivariate Pearson's analysis.

Results: In SSc pts the percentage of circulating M2 cells (CD14⁺CD204⁺CD163⁺CD206⁺ cells) was significantly increased compared to both HSs and SSc pts not under immunosuppressive treatment ($p<0.05$). However, no correlation with skin involvement and SSc-related antibodies was observed. Cultured macrophages stimulated with SSc serum expressed CD204 and CD206 markers compared to the macrophages stimulated with HS serum (CD204 and CD206 double negative cells). Of note, the ability to express M2 markers was already evident in cultured macrophages stimulated with "Early" NVC SSc serum and their expression even increased in macrophages stimulated with "Active" and "Late" NVC sera together with the secretion of MMP9. Same results were observed also in cultured THP1-derived macrophages.

Conclusion: The study confirmed that SSc pts are characterized by a significant increase of circulating M2 cells, suggesting their possible involvement in the pathogenesis of the disease. Interestingly, results insinuate that sera from SSc patients already in an "Early" NVC condition (sera known to contain specific profibrotic molecules such as cytokines, growth factors like TGF β 1 or endothelin-1) seem able to induce *in vitro* a profibrotic M2 macrophage phenotype.

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