time of GAVE occurrence and the last observation was collected to define the outcomes.
Results. 49 cases of SSc patients with GAVE were included (24 with diffuse cutaneous SSc) and compared to 93 SSc controls. The prevalence of GAVE was estimated at about 1% of SSc patients. By multivariate analysis, SSc-GAVE patients exhibited more frequently a diminished (<75%) DLCO value (Odds Ratio, OR : 12.8; 95% confidence interval, CI, 1.9-82.8) despite less frequent pulmonary fibrosis (OR : 0.2; 95%, CI 0.1-0.6). GAVE was also associated with the presence of anti-RNA-polymerase III antibodies (OR : 4.6; 95%CI 1.2-21.1). SSc-GAVE was associated with anemia (82%) requiring blood transfusion (45%). Therapeutic endoscopic procedures were performed in 45% of GAVE cases. After a median follow-up of 30 months (range 1-113 months), survival was similar in SSc-GAVE patients, as compared to controls but a higher number of scleroderma renal crisis occurred (12% vs. 2%. p=0.01).
Conclusion. GAVE is rare and associated with a vascular phenotype including anti-RNA-polymerase III antibodies and a high risk of renal crisis. Anemia usually requiring blood transfusions is a common complication.

Session 5: *Links to Inflammation, Immunity and Vasclular Disease*

S.5.1

IMMUNOLOGICAL MECHANISMS OF FIBROSIS

T.A. Wynn
Program in Tissue Immunity and Repair, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, USA.

Macrophages are found in close proximity with collagen-producing myofibroblasts and play key roles in the mechanisms of wound healing and fibrosis. They produce growth factors and pro-fibrotic mediators that directly activate fibroblasts, including transforming growth factor beta, insulin-like growth factor, vascular endothelial growth factor, and platelet-derived growth factor. They also regulate extracellular matrix turnover by influencing the balance of various matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases. Macrophages also regulate fibrogenesis by secreting chemokines that recruit fibroblasts and other inflammatory cells and by producing various inflammatory and anti-inflammatory cytokines. With their potential to act in both a pro- and anti-fibrotic capacity at distinct stages of the wound healing response, macrophages and the factors they express are integrated into all stages of the fibrotic process. These various and sometimes opposing functions are performed by distinct macrophage subpopulations, the identification of which is a growing focus of fibrosis research. Although collagen-secreting myofibroblasts once were thought of as the master ''mediators" of fibrosis, in this presentation I will illustrate how macrophages function as the master ''regulators" of fibrosis.

S.5.2

PIGMENT EPITHELIUM DERIVED FACTOR SECRETED BY SSC FIBROBLASTS INHIBITS ANGIO AND VASCULOGENESIS IN VITRO

V. Liakouli[1,2], G. Mavria[3], J. Gillespie[1], M. Scarcia[3], P. Cipriani[2], R. Giacomelli[2], P. Emery[1,4], F. Del Galdo[1,4]
[1]Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds, Leeds, UNITED KINGDOM; [2]Department of Biotechnological and Applied Clinical Science, Rheumatology Unit, University of L'Aquila, L'Aquila, ITALY; [3]Signal Transduction and Angiogenesis group, Leeds Institute of Cancer and Pathology, University of Leeds, Leeds, UNITED KINGDOM; [4]NIHR Leeds Musculoskeletal Biomedical Researc Unit, Leeds Teaching Hospital NHS Trust, Leeds, UNITED KINGDOM.

Background. Systemic Sclerosis (SSc) is an autoimmune disorder characterized by tissue fibrosis and defective angio/vasculogenesis. There is scanty of studies investigating the molecular mechanisms linking the two processes in SSc. Recently, a proteomic analysis of SSc dermal fibroblasts (SScFBs) secretome, identified an increased secretion of Pigment Epithelium Derived Factor (PEDF) compared to healthy fibroblasts. PEDF produced by retinal-pigmented epithelium and melanocytes (HEMs), is the major endogenous inhibitor of intraocular angiogenesis. Here we aimed to validate the increased expression of PEDF in SSc and to determine whether PEDF might play a role in SSc vasculopathy.
Methods. PEDF expression was investigated in the involved skin and FBs of 4 early diffuse SSc patients and 4 healthy controls (HC) by immunohistochemistry (IHC) and rt-PCR. Functional effects of PEDF on angio/vasculogenesis were examined by Matrigel assays and organotypic co-culture assays of HUVECs or microvascular endothelial cells (MVECs), on either primary healthy FBs (HCFBs) or SScFBs or HCFBs silenced for Caveolin-1 (Cav-1). Endothelial cells were visualized by CD31 staining. Vascular tubule number, length and junctions were analyzed by Angiosys software (TCS CellWorks).
Results. In SSc skin 52% (+/-5.9) of dermal fibroblasts were positive for PEDF vs. 13% (+/-0.68) of FBs in HC skin ($p<0.05$). Furthermore, double IHC studies indicated that PEDF positive FBs showed a decreased Cav-1 expression in both HC and SSc skin. In-vitro studies confirmed that SScFBs showed on average a 5-fold increased PEDF expression when compared to HCFBs ($p<0.0162$). Additionally, consistent with IHC studies HCFBs silenced for Caveolin-1 showed on average a 2-fold increase in PEDF mRNA levels compared to control ($p<0.0055$). Matrigel studies indicated that recombinant PEDF protein inhibited vasculogenesis, suppressing the loop number by 20% ($p<0.05$). Consistently, co-culture assays indicated that PEDF inhibited tubulogenesis, suppressing both total tubule length by 42% ($p<0.005$), number of tubules by 55% ($p<0.005$) and

junctions by 73% (p<0.001). Importantly, co-culture assays indicated that primary SScFBs inhibited tubulogenesis on MVECs. Additionally, HCFBs silenced for Caveolin-1 inhibited HUVECs tubulogenesis, suppressing both total tubule length by 63% (p<0.001), number of tubules by 61% (p<0.001) and junctions by 88% (p<0.001).
Conclusion. The increased expression of PEDF in SSc may be secondary to loss of Caveolin in dermal fibroblasts and contribute to the vascular manifestation of Scleroderma. Further studies unraveling the mechanisms of the antiangiogenic effect of PEDF may shed light in understanding the molecular events linking the profibrotic phenotype and SSc vasculopathy.

S.5.3

SCLERODERMA DERMAL FIBROBLASTS OVEREXPRESS VASCULAR ENDOTHELIAL GROWTH FACTOR DUE TO AUTOCRINE TRANSFORMING GROWTH FACTOR BETA SIGNALING

I. Kajihara, M. Jinnin, N. Honda, K. Makino, T. Makino, S. Fukushima, H. Ihn
Kumamoto University, Kumamoto, JAPAN

Objective. Overexpression of vascular endothelial growth factor (VEGF) in scleroderma (SSc) skin may play a role in the pathogenesis of the disease. Our study was undertaken to evaluate whether dermal fibroblasts function as one of the sources of the increased VEGF in SSc, and to clarify its mechanism.
Methods. Protein and mRNA levels of VEGF were analyzed using immunoblotting, enzyme-linked immunosorbent assay, and real-time PCR. The DNA-binding ability of Smad3 was evaluated by DNA affinity precipitation.
Results. VEGF mRNA expression in vivo was increased in SSc skin compared to skin with other collagen diseases. Expression of VEGF protein and mRNA in cultured SSc dermal fibroblasts was constitutively and significantly upregulated. Ectopic TGF-β stimulation induced VEGF synthesis in normal fibroblasts, and TGF-β knockdown normalized the upregulated VEGF levels in SSc fibroblasts. Furthermore, Smad3 overexpression induced VEGF levels. We found that bp -532 to -521 on the VEGF promoter is a putative binding site for Smads, and that the binding activity of Smad3 to VEGF promoter was constitutively increased in SSc fibroblasts as well as in normal fibroblasts treated with exogenous TGF-β1.
Conclusions. We demonstrated that VEGF were overexpressed due to autocrine TGF-β/Smad signaling in SSc. TGF-β signaling may contribute to the pathogenesis of angiopathy as well as tissue fibrosis.

S.5.4

IL6 TRANS-SIGNALLING AND CCL2 CO-REGULATE FIBROBLAST DEPENDENT TRANS-ENDOTHELIAL MIGRATION OF MONONUCLEAR CELLS AND FIBROTIC RESPONSE IN SCLERODERMA

R. Alade, K. Khan, S. Xu, C. Denton, V. Ong
Centre for Rheumatology and Connective Tissue Diseases, UCL Medical School Royal Free Campus, London, UNITED KINGDOM.

Background. IL6 is a key mediator implicated in activation of extracellular matrix (ECM) in scleroderma (SSc) fibroblasts. CCL2 is a proinflammatory chemokine that is overexpressed in diffuse cutaneous systemic sclerosis (dcSSc). We explored interaction between these two mediators and their role in the recruitment of inflammatory cells and ECM production. Methods: Dermal fibroblasts were cultured from skin biopsies from healthy controls (n=4) and early stage dcSSc (n=4). Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples of the latter group. Induction of CCL2 by IL6 via trans-signalling in dermal fibroblasts and the effect of SSc fibroblast-derived CCL2 on migration of PBMCs across an endothelial layer in vitro were studied using Transwell migration assays in a co-culture system. The effect of PBMC-fibroblast cross-talk on induction of ECM proteins: α -smooth muscle actin (α SMA) and Collagen type-I (Col-I) was assessed by neutralising antibodies against CCL2 or IL6 receptor and targeted ectodomain shedding inhibition using TNF-α processing inhibitor-1(TAPI-1).
Results. IL6 trans-signalling increased CCL2 expression (mean ± SEM % basal expression) (33± 2.7% p<0.03 and 45±5.6% p<0.04) in control and SSc fibroblasts respectively. CCL2 expression was reduced in the presence of anti-IL6R in control fibroblasts (63±6.4%, p<0.04). IL6 trans-signalling increased migration of PBMCs (n=4) by 2.1 fold (p<0.05) and 4.5 fold (p<0.03) in the presence of control fibroblasts and SSc fibroblasts respectively. The migration of PBMCs was significantly reduced by the addition of neutralising antibodies against CCL2 and IL6R (44± 5.1%, p<0.05 and 62±5.4%, p=0.04) respectively and both antibodies combined (44±7.3.2%, p<0.05) in the presence of SSc fibroblasts. In response to IL-6 trans-signalling, there was increased expression of α SMA (53 ± 5.9%, p<0.04) and Col-I (70±2.6%, p<0.03) at 24-hour in the presence control fibroblasts and α SMA (37± 5.9%, p<0.03) and Col-I (47±3.6%, p<0.04) in the presence of SSc fibroblasts. TAPI-1 reduced PBMC migration in a concentration dependent manner with maximal effect at 50μM by (55±4.1 % p<0.04) and TAPI-1 reduced synthesis of α SMA (27± 4.8 %, p<0.05) and Col-I (31±3.6%, p<0.03) respectively.
Conclusions. Our data suggest that fibroblast-derived CCL2 expression is regulated by IL-6 via trans-signalling. The IL-6/CCL2 interplay regulates trans-endothelial migration of PBMCs and IL-6 trans-signalling with intramembrane shedding of IL-6R mediates the fibrotic response. Thus, CCL2/IL6 interplay may be important in SSc pathogenesis and could be targeted therapeutically.

S.5.5

THE GLOBAL MICRORNA PROFILE OF SKIN IN SYSTEMIC SCLEROSIS

G. Salazar, J. Hagan, M. Wu, M. Mayes, S. Assassi
University of Texas Health Science Center at Houston, Houston, USA

Background. The underlying pathogenesis of systemic sclerosis (SSc) remains poorly understood contributing to limited efficacy of therapeutic options.
MicroRNAs (miRNAs) are small non-coding RNAs that play an important role in post-transcriptional gene regulation. Two families of dysregulated miRNAs, miR-21 and miR-29, have been implicated in the pathogenesis of fibrotic diseases and replicated in SSc. These studies focused on a miRNA of interest and global skin miRNA profiling in SSc has not been reported.
Recent advances in quantitative polymerase chain reaction (qPCR) allow simultaneous measurement of hundreds of miRNAs. The objective of this study was to use this technology to identify the unbiased, global miRNA profiling of SSc skin and evaluate their potential role in its pathogenesis.
Methods. We investigated the miRNA profile in SSc skin compared to unaffected controls using multiplex qPCR platform. We obtained forearm skin samples (3 mm punch biopsy) from 10 patients with early SSc (<5 yrs, on no immunosuppression) and 10 age-, gender- and ethnicity matched controls. Total RNA was isolated using Qiagen miRNAeasy mini kit and examined by Exiqon LNA-enhanced (locked nucleic acid) miRNA qPCR. Levels of 752 miRNAs were determined. Unsupervised hierarchical clustering analysis was performed. Patient and control sample miRNA levels were compared and differences with a p<0.01, false discovery rate (FDR) <10% and fold change >2 were considered statistically significant.
Results. The unsupervised hierarchical clustering analysis showed that the miRNA skin profile almost perfectly separated SSc patients and controls. Only one patient clustered along with controls (Figure 1). Comparison of patient to control samples revealed 26 miRNAs that were differentially expressed. Eighteen of these (69%) were part of the largest known human miRNA cluster (miR-379/miR-656) located on chromosome 14q32.3. Three miRNAs were encoded in a cluster on chromosome X (Xq26.3). We confirmed the previously reported up-regulation of miR21-5p in SSc.
Conclusions. To our knowledge, this is the first global, unbiased examination of miRNAs in SSc skin. The miRNA profile almost perfectly separated SSc patients and controls. We observed 26 dysregulated miRNAs, most of them coming from two clusters, one of them located in chromosome X. This finding might have important biological implications considering the female predilection of SSc. Dysregulation of these miRNA clusters have not been reported in SSc and other autoimmune diseases. The results of our study link miRNA to the pathogenesis of SSc and could have important ramifications for future drug and biomarker development.

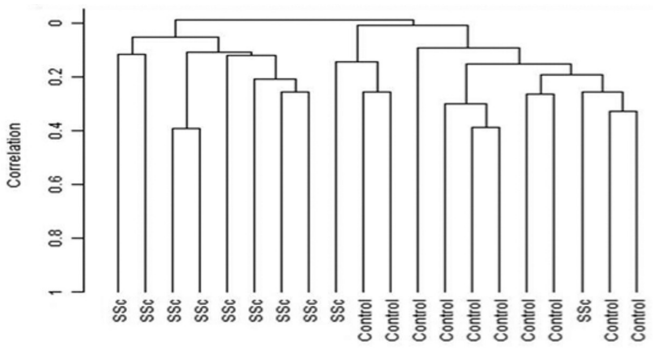

Fig. 1. Unsupervised clustering of miR profiles observed in patient and control skin samples.

S.5.6

THE PRESENCE OF A COLD TEMPERATURE SENSOR IN THE VASCULAR ENDOTHELIUM: ENHANCED EXPRESSION IN SSc SKIN AND ENDOTHELIAL CELLS DYSFUNCTION AFTER ACTIVATION

B. Kahaleh, D. Giovannucci, Y. Wang
University of Toledo, Department of Rheumatology, Toledo, USA

Background. Cold exposure results in severe vasospasm and reperfusion vascular injury in SSc. The mechanisms responsible for enhanced cold sensitivity in SSc are poorly understood. Transient Receptor Potential Melastatin 8 (TRPM8) is a known cold sensing cation channel receptor. To date, TRPM8 expression has not been characterized in microvascular endothelial cells (MVEC). In this study we thought to investigate TRPM8 expression in normal and SSc MVEC and skin. We also investigated the effects of TRPM8 activation on MVEC gene expression.
Methods. MVEC were isolated from involved SSc skin and from matched healthy control subjects. The expression of TRPM8 was determined by RT-PCR, immunohistochemistry and by western blot analysis. TRPM8 activation was triggered by the addition of the agonist menthol or by exposure to cold temperature (18C°). The intracellular calcium concentration was determined by Ca2+ microfluorometry. The expression levels of TRPM8 in SSc-MVEC and SSc skin biopsies and the effects of TRPM8 activation on MVEC mRNA expression of ET1, NOS3 and PTGIS were determined by real time PCR.
Results. TRPM8 gene and protein expression in MVEC were confirmed by RT-PCR, Western blotting and immunohistochemistry. MVEC intracellular calcium ([Ca2+]i) influx into the cells in response to the addition of TRPM8 agonist menthol are demonstrated by Ca2+ microfluorometry studies. The activation of TRPM8 in MVEC by cold temperature or by menthol significantly increased the expression of ET1 (2.4 folds ± 0.21) and decreased NOS3 (62% ± 5.1 reduction) and PTGIS (61% ± 4.8) expression levels. These effects were reversed by the addition of the TRPM8 antagonist capsazepine. TRPM8 mRNA expression levels were significantly increased in SSc-MVEC (2.6 fold ± 0.22 vs. control MVEC) and SSc-skin biopsies (5.5 fold ± 2.3 vs. control skin biopsies).
Conclusions. The study demonstrates that human MVEC express functional TRPM8 and that there is increased expression of TRPM8 in SSc skin and in SSc-MVEC. TRPM8 may be involved in cold-induced vascular dysfunction through increase ET1, and decrease the NOS3 and PTGIS mRNA expression. The increased expression levels of TRPM8 in SSc-MVECs and SSc skin may mediate the known enhanced cold sensitivity in SSc. These results suggest that the blockade of TRPM8 activation could be an effective therapeutic strategy in SSc vasculopathy.

Session 7: *Treatment and DMARDS in SSc*

S.7.1

HOW TO TREAT RAPIDLY PROGRESSIVE SSc

C.P. Denton
Royal Free Hospital and UCL Medical School, London, UNITED KINGDOM

Systemic sclerosis (scleroderma; SSc) has a high mortality and morbidity but varies widely in rate of disease progression, reflecting clinical heterogeneity and disease subset. The most rapidly progressive cases are usually those with diffuse skin involvement and typically the maximum rate of progression is within the first 3 to 5 years of disease onset. The rate of change in skin scleroiss score can be assessed and has been associated with increased risk of major complications including cardiac involvement, lung fibrosis or scleroderma renal crisis. In many cases progression occurs over the first few months of disease and is associated with swelling and skin thickening affecting the distal limbs, pruritis over the proximal skin and the presence of tendon friction rubs on clinical examination. This constellation of signs is recognised to put a patient at high risk of scleroderma renal crisis and vigilant observation and patient education is important to minimise the delay in diagnosing this treatable complication that previously had very high mortality. Elevated ESR, platelet count and CRP are also recognised as markers of disease activity and poor outcome. In addition to skin progression there is risk of lung fibrosis and serious cardiac involvement with systolic impairment and cardiac arrhythmias. Thus treatment for SSc at this stage should include supportive management of manifestations such as Raynaud's phenomenon and reflux oesophagitis and investigation for major organ based pathology. Severe skin disease or presence of cardiac or lung fibrosis my require treatment with intravenous cyclophosphamide followed by maintenance immunosuppression with mycophenolate mofetil or methotrexate although less severe cases may be treated initially with these oral drugs, reserving cyclophosphamide for more severe or refractory patients. Finally, there are emerging data supporting the potential value of HSCT in this group. The challenge is case selection as the ASTIS trial suggests a potential treatment related mortality of up to 10% although long term survival sand disease burden may be significantly improved. Cases with cardiac involvement, pulmonary hypertension and smokers may be especially at risk of TRM and are probably not suitable cases for this treatment despite their poor overall prognosis with standard therapy. Autoantibody reactivity may be especially helpful in identifying cases of rapidly progressive diffuse SSc as the anti-RNA polymerase III and anti-U3RNP positive patients are more often in this group and ANA patterns can eb defined early in the disease. This is important considering the emphasis on early diagnosis that will identify milder cases of SSc and so predictors of rapid progression are especially valuable.