MSCs transplantation. PBMCs from 8 patients were collected and co-cultured with UC MSCs at ratios of 1:1, 10:1 and 50:1, for 24 hours, 48 hours and 72 hours, respectively, to detect the levels of tolerogenic DCs. The FLT3L in the supernatant solution were determined. FLT3L siRNA was added to the co-culture system, and the level of tolerogenic DCs were detected.
Results: The levels of peripheral $CD1c^+$ DCs and serum FLT3L were significantly decreased in SLE patients compared to healthy controls. After UC MSCs transplantation, the levels of $CD1c^+$ DCs increased, along with an increase in serum FLT3L. In vitro studies showed that UC MSCs time-dependently up-regulated peripheral $CD1c^+$ DCs, but not dose-dependently. The supernatant FLT3L level significantly increased after co-cultured with MSCs. However, the addition of FLT3L siRNA significantly abrogated the up-regulation of $CD1c^+$ DCs by MSCs.
Conclusions: UC MSCs induce $CD1c^+$ tolerogenic DCs through up-regulating FLT3L in lupus patients.
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.3334

Scleroderma, myositis and related syndromes - etiology, pathogenesis and animal models

AB0164 AMYLOIDOSIS IN PROGRESSIVE SYSTEMIC SCLEROSIS – A POSTMORTEM CLINICOPATHOLOGIC STUDY OF 12 PATIENTS

Á. Apáthy[1], M. Bély[2]. *[1]Department of Rheumatology, St. Margaret Clinic Budapest; [2]Department of Pathology, Hospital of the Order of the Brothers of Saint John of God, Budapest, Hungary*

Background: Systemic sclerosis (SSc), like all chronic autoimmune disorders, may be complicated by AA amyloidosis (AAa). An associated B-cell lymphoma my cause amyloid λ or κ light-chain deposition and AL amyloidosis (ALa) in SSc as well.
Objectives: The aim of this study was to determine the prevalence and type of amyloidosis in SSc patients, appraise the extent of amyloid deposits in various organs.
Methods: 12 patients (females 11, age: 54.82 years, range 66–32, onset of SSc: 48.86, disease duration: 6.43 years; one male, age: 65.0 years at death, onset of SSc and disease duration not known) were studied. All patients were autopsied. SSc was diagnosed clinically according to the criteria of the ACR [1].
Amyloid deposits on different tissue structures [arteriole, small artery, medium size artery, venule, small vein, medium size vein, interstitial collagen fiber, reticulin fiber (collagen IV), basal lamina, nerve, renal glomerulus] of 6 organs [heart, lungs, kidney, gastrointestinal tract, skin and brain] were determined histologically. The extent of amyloid A deposits was evaluated by semi-quantitative, visual estimation on a 0 to 3 plus scale, based on the number of involved tissue structures per light microscopic field [2] ("0": no amyloid deposits, "1": Sporadic, minimal amyloid deposits on different tissue structures, "2": less than five, "3": five or more involved tissue structures per microscopic field at objective magnification of x20)
The prevalence and extent of amyloid deposits in various organs were compared by Student (Welch) t-probe.
Results: Systemic AL-l light-chain amyloidosis was diagnosed in 1 (8.0%) 67 year old female patient (onset of SSc: 66 years, disease duration 1 year), and systemic AAa in 1 (8.0%) 53 year old female patient (onset of SSc: 41 years, disease duration 12 years).
The prevalence (in %) and the average extent of AL-l light-chain and amyloid A deposits (absolute value) in various organs of SSc patients are summarized in Table 1.

Tab;e 1

Organs	SSc-λ Prevalence in %	SSc-AAa Prevalence in %	p<	SSc-λ Average extent	SSc-AAa Average extent	p<
Skin	80.00	0.00	**0.0001**	1.45	0.00	**0.0002**
Heart	72.73	63.64	0.3329	1.45	0.91	0.117
Kidney	63.64	63.64	0.1228	1.09	0.73	0.185
G-I Tract	41.67	66.67	0.1185	1.08	1.00	0.433
Lung	40.00	40.00	0.5000	1.00	0.50	0.077
Brain	0.00	0.00		0.00	0.00	–
Average/Organ	50.79	41.27	**0.052**	1.040	0.556	**0.0036**
Average/Patient	45.56	35.96	0.144	0.965	0.494	**0.0006**

Conclusions: AL-λ deposits were present earlier and were more prominent in the skin, heart and kidney, than in the, G-I tract and the lung. Amyloid A deposits were present earlier and were more prominent in the G-I tract, heart and kidney, than in the, lung and the skin. In the brain AL-λ and amyloid A deposits were absent in both diseases.
Higher prevalence (80.0% versus 0.0%; $p<0.0001$) and massive AL-λ deposition in the skin (1.45 versus 0.0; $p<0.0002$) may be explained by qualitative differences of AL-λ and amyloid A and by a diverse affinity of circulating amyloid precursors to the qualitative changed interstitial collagen and reticulin fibers. In systemic sclerosis patients qualitative change of collagens has been demonstrated [3–5].

References:
[1] van den Hoogen F et al: Ann Rheum Dis 2013; 72:1747–1755.
[2] Bély M, Apáthy Á: Clinical Pathology of rheumatoid arthritis. Akadémiai Kiadó, Budapest 2012 http://www.akkrt.hu.
[3] Istok R, et al. Annals Rheum Dis 1999; 58(Suppl1): 192.
[4] Istok R, et al: Reumatologia 2000; 2: 91.
[5] Istok R, et al: Exp Dermatol 2001; 26:545–547.
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.1226

AB0165 CXCL4 MAY PLAY A KEY ROLE IN SYSTEMIC SCLEROSIS BY DRIVING CD4 T CELLS TO PRODUCE IL-17

A.J. Affandi[1,2], S.C. Silva Cardoso[1,2], S. Garcia-Perez[1,2], W. Marut[1,2], J.A.G. van Roon[1,2], T.R.D.J. Radstake[1,2]. *[1]Laboratory of Translational Immunology; [2]Dept. of Rheumatology & Clinical Immunology, UMC Utrecht, Utrecht, Netherlands*

Background: CXCL4 regulates multiple facets of immune response and its level is highly increased in various Th17-associated rheumatic diseases, including systemic sclerosis (SSc)[1–4]. Recently, CXCL4 was shown to induce type I interferon production as well as endothelial activation in SSc patients[1]. Th17 skewing has been demonstrated in SSc[5–6], however, whether CXCL4 plays a role in the induction of IL-17 production by human CD4 T cells is currently unclear.
Objectives: To investigate the effect of CXCL4 on human CD4 T cell phenotype in particular IL-17 production in the absence or presence of antigen presenting cells.
Methods: Blood was obtained from healthy donors and CD4 T cells, monocytes, dendritic cells, were isolated using magnetic-based sorting (n=20). In addition, CD4 T cells from SSc patients were isolated (n=10). CD4 T cells were activated using anti-CD3/CD28, or in co-cultures with antigen presenting cells, stimulated using superantigen Staphylococcal enterotoxin B. Exogenous recombinant human CXCL4 was added during (co-)culture in different concentrations. Cytokine production and proliferation were analyzed using Luminex immunoassays, intracellular cytokine staining, and flow cytometry.
Results: CXCL4 directly induced CD4 T cells secreting both IL-17 and IFN-γ, as well as IL-22, when costimulated with anti-CD3/CD28 ($p<0.05$). In many SSc patients, similar IL-17 increase upon CXCL4 treatment was observed, although this did not reach statistical significance. This might be due to the fact that CD4 T cells from SSc patients had already significant higher levels of IL-17 as compared to healthy donors (2182±722.2 vs 1053±263.6 pg/ml, mean±SEM, $p<0.05$). Furthermore, in co-culture system of CD4 T cells with monocytes or myeloid dendritic cells, CXCL4 treatment induced IL-17 production upon triggering by superantigen ($p<0.05$). Moreover, when monocyte-derived dendritic cells were differentiated in the presence of CXCL4, they orchestrated significantly increased levels of IL-17, IFN-γ, and proliferation by CD4 T cells (all $p<0.05$).
Conclusions: Altogether, we demonstrate that CXCL4 boosts pro-inflammatory cytokine production especially IL-17 by human CD4 T cells, either by acting directly or indirectly via antigen presenting cells. This indicates that targeting CXCL4 may potentially alleviate immune responses in Th17-mediated rheumatic diseases such as systemic sclerosis.
References:
[1] van Bon L, Affandi AJ, Broen J, et al. N Engl J Med 2014.
[2] Tamagawa-Mineoka R, et al. Allergol Int 2008.
[3] Vettori S, et al. Clin Rheumatol 2016.
[4] Yeo L, et al. Ann Rheum Dis 2015.
[5] Radstake TRDJ, van Bon L, et al. PLoS One 2009.
[6] Yang X, et al. Arthritis Res Ther 2014.
Acknowledgements: This study was supported by the Dutch Arthritis Association (Reumafonds) to A.J.A. and T.R.D.J.R., the Netherlands Organization for Scientific Research (NWO) to A.J.A. (Mosaic grant 017.008.014) and T.R.D.J.R. (Pre-Seed grant), the Portuguese Fundação para a Ciência e a Tecnologia to S.C.S.C. (SFRH/BD/89643/2012) and the European Research Council to T.R.D.J.R. (ERC Starting grant).
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.6100

AB0166 INTERLEUKIN-6-POLARISED MACROPHAGES PROMOTE DERMAL MYOFIBROBLAST DIFFERENTIATION

A. Tam, X. Shi Wen, B. Ahmed-Abdi, C. Denton, D. Abraham, V. Ong. *University College London, London, United Kingdom*

Background: Macrophages and fibroblasts are key effector cell types present in scleroderma tissue[1]. While the effect of scleroderma fibroblast conditioned medium on macrophages has been previously studied[2], less is known about the effect of scleroderma macrophages on fibroblasts. Interleukin-6 (IL-6) is an important mediator of fibrosis and is overexpressed in scleroderma sera and cells such as skin fibroblasts, monocytes and endothelial cells[3]. Given the increase in IL-6 levels in scleroderma and presence of the IL-6 receptor on the cell surface of macrophages, we are now investigating the phenotype of macrophages exposed to IL-6. Here we present our work into the paracrine function of IL-6-treated macrophages in stimulating fibroblasts using a media transfer approach.

Objectives: To investigate the effect of macrophage conditioned media on fibroblast activation.
Methods: PBMC-derived macrophages from healthy control (n=3 females, mean age 50.8±21.9 years) and diffuse scleroderma (n=4 females, mean age 54.8±15.7 years, mean disease duration 73.2±90.3 months, 2 with antiScl70 and 2 with antiRNA polymerase antibodies) individuals were cultured in RPMI/10% FBS/M-CSF (4ng/ml)/P/S, quiesced in media with 1% BSA replacing the FBS, and left untreated (M0) or treated with IL-6 (50ng/ml, M (IL-6)) for 24 hours. The cultures were replaced with fresh media and collected after 24 hours. Conditioned media were applied to healthy control skin fibroblasts (24 hours) and fibroblast expression of fibrotic proteins was assessed by Western Blot, using β-tublin and TBP as loading controls. As control, fibroblasts from healthy volunteers were left untreated by culturing in non-conditioned media (fresh RPMI/ 1% BSA/M-CSF (4ng/ml)/P/S).
Results: Fibroblast expression of collagen type I and connective tissue growth factor (CTGF) were not significantly different between untreated and macrophage conditioned medium treatment groups. Baseline levels of collagen type I were high in the fibroblasts cultured in non-conditioned media, and there was a trend towards increased CTGF expression in all conditioned media-treated groups compared to untreated fibroblasts in non-conditioned media. A 2.6-fold increase in α-smooth muscle actin (α-SMA) was observed in the healthy control M (IL-6)-conditioned medium-treated group compared to the group of fibroblasts cultured in non-conditioned media (one-way ANOVA with Sidak multiple comparison, p=0.048).
Conclusions: After 24 hours treatment, control dermal fibroblasts treated with media of IL-6-polarised healthy control macrophages expressed higher levels of α-SMA compared to fibroblasts cultured in non-conditioned medium. A trend towards increased CTGF was also observed. These results suggest that paracrine factors in the IL-6-activated macrophage secretome may promote differentiation of fibroblasts into myofibroblasts, which is a key component of wound healing and scleroderma fibrosis.
References:
[1] Fuschiotti P. Current perspectives on the immunopathogenesis of systemic sclerosis. Immunotargets Ther. 2016;5:21–35.
[2] Denton CP, Shi-Wen X, Sutton A, et al. Scleroderma fibroblasts promote migration of mononuclear leucocytes across endothelial cell monolayers. Clin Exp Immunol. 1998;114:293–300.
[3] Khan K, Xu S, Nihtyanova S, et al. Clinical and pathological significance of interleukin 6 overexpression in systemic sclerosis. Ann Rheum Dis. 2012;71:1235–42.
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.4868

AB0167 CALCIUM INFLUX KINETICS AND THE CHARACTERISTICS OF POTASSIUM CHANNELS IN PERIPHERAL T LYMPHOCYTES IN SYSTEMIC SCLEROSIS

N. Legány[1], G. Toldi[2], I. Ocsovszki[3], L. Kovács[1], A. Balog[1]. *[1]Department of Rheumatology and Immunology, Faculty of Medicine, Albert Szent-Györgyi Health Center, University of Szeged, Szeged; [2]First Department of Obstetrics and Gynecology, Semmelweis University, Budapest; [3]Department of Biochemistry, Faculty of Medicine, Albert Szent-Györgyi Health Center, University of Szeged, Szeged, Hungary*

Background: Systemic sclerosis (SSc) is a chronic connective tissue disorder characterized by microvascular injury, fibrosis and autoimmunity that affects the skin and internal organs. The short-term activation of peripheral blood T lymphocytes plays a crucial role in initiating and maintaining the chronic inflammation. The transient increase of the cytoplasmic free calcium level plays a key role in the process of lymphocyte activation. Kv1.3 and IKCa1 potassium channels are important regulators of the maintenance of calcium influx during lymphocyte activation. The influx of calcium is maintained by the function of potassium channels that conserve the electrochemical potential gradient via the efflux of potassium from the cytoplasm. Recent reports raised the notion that the inhibition of lymphocyte potassium channels, especially that of the Kv1.3 channel would be an straightforward solution for specific immunosuppression in autoimmune disorders. Furthermore, our previous studies described an alteration of the short-term activation of peripheral lymphocytes in rheumatoid arthritis and primary Sjögren's syndrome (pSS), and the overexpression of Kv1.3 channels in pSS.
Objectives: Therefore, in this study we aimed to characterize the effects of lymphocyte potassium channel inhibition on short-term peripheral blood T lymphocyte activation in major lymphocyte subsets in SSc.
Methods: We enrolled 12 healthy individuals and 16 SSc patients. We evaluated calcium influx kinetics following activation in CD4, Th1, Th2 and CD8 cells applying a novel kinetic flow cytometry approach. We assessed the sensitivity of the above subsets to specific inhibition of the Kv1.3 and IKCa1 potassium channels. We also assessed the Kv1.3 expression on lymphocytes.
Results: We observed increased parameters of calcium influx in CD8+ lymphocytes' as compared with Th1 cells in SSc. However, the activation of CD8+ cells was lower in SSc compared to healthy controls. Moreover, activation of Th1 lymphocytes was slower in SSc than in healthy controls. The inhibition of IKCa1 potassium channel decreased the activation of CD8+ lymphocytes in healthy controls and the activation of Th1 cells in SSc. The inhibition of Kv1.3 channel modified the dynamics of activation of Th1 and Th2 lymphocytes in SSc.
Conclusions: The altered function of CD8+ cells and the specific inhibition of potassium channels seem to be a consequence of altered calcium influx kinetics in SSc, distinguishing it both from healthy controls and other autoimmune diseases.
Acknowledgements: Attila Balog and Gergely Toldi were supported by the János Bolyai Scholarship. Gergely Toldi is an International Society for the Advanecement of Cytometry (ISAC) Scholar.
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.5310

AB0168 PROTECTIVE EFFECTS OF EPIGALLOCATECHIN 3 GALLATE ON FIBROSIS IN SCLERODERMA MODEL

A. Kocak[1], D. Harmancı[1], M. Birlik[2], S. Sarioglu[3], G. Guner Akdogan[4]. *[1]Department of Molecular Medicine, Institute of Health Sciences, Dokuz Eylul University; [2]Department of Internal Medicine, Division of Rheumatology & Immunology; [3]Department of Medical Pathology, School of Medicine, Dokuz Eylul University; [4]Department of Biochemistry, School of Medicine, Izmir University of Economics, Izmir, Turkey*

Background: Scleroderma (SSc) is a disease that shows involvement in internal organs or on the skin characterized by fibrosis (1). Dermis thickening and uncontrolled extracellular matrix (ECM) increase are seen in this disease whose pathogenesis is not fully understood. TGF-β/Smad 2&3 pathway is pivotal role in SSc pathogenesis via induction of profibrotic molecules including collagen and by decrease of matrix metalloproteinases (MMPs) synthesis (2,3). The occurrence of the myofibroblast phenotype at fibrosis is thought to be responsible for the contracted regions of the affected tissues (4).
Objectives: The aim of this study with bleomycin (BLM) formed in an experimental model of scleroderma is to investigate the potential effects of epigallocatechin-3-gallate (EGCG) against fibrosis.
Methods: 32 *Balb/c* female mice were randomly selected into four groups.For 21 days: (1) Control group (n: 8) was given 100 μ L *subcutan (sc)* saline (SF) once a day, 100 μL *intraperitoneal (ip)* SF twice a week, (2) BLM group (n: 8) was given 100 μL (100 ug) *sc* BLM once a day, 100 μL *ip* SF twice a week, (3) BLM + EGCG group (n:8) was given 100 μL (100 ug) *sc* BLM once a day, 100 μL (100 μg) *ip* EGCG twice a week, (4) EGCG group (n: 8) was given 100 μL *sc* SF once a day, 100 mL (100 μg) *ip* ECCG twice a week. Hematoxylin&eosin and Masson trichrome staining of dermal areas were performed. Myofibroblast activity was measured using alpha smooth muscle actin antibody (αSMA) by immunohistochemistry.Expression levels of MMP-1, MMP-8, MMP-13 and p-SMAD protein were examined by western blot. Expression levels of TGF-β mRNA were examined by qPCR. All of the statistical analyses were performed using SPSS software and the quantitative data were expressed as the means±SEM.The quantitative variables were compared using the a ANOVA-Sidak. Statistical significance was defined as $p<0.05$

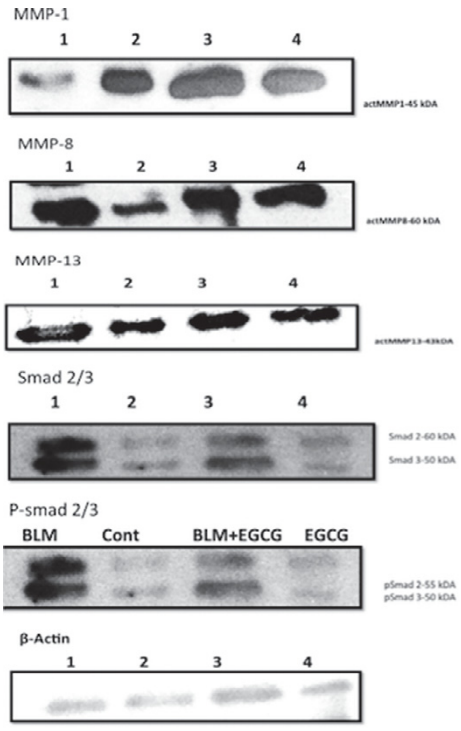

Figure 5. MMP-1, MMP-8, MMP-13, SMAD 2/3 and p-SMAD2/3 expression. β- Actin used for internal control.