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.
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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.
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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 $p<0.05$

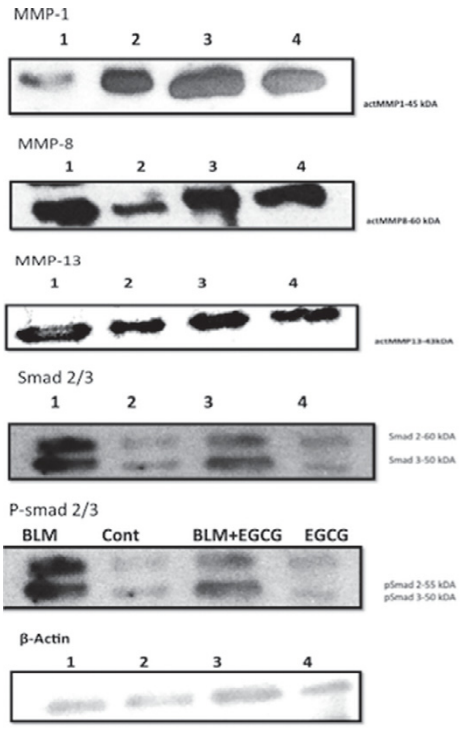

Figure 5. MMP-1, MMP-8, MMP-13, SMAD 2/3 and p-SMAD2/3 expression. β- Actin used for internal control.