thickness and collagen accumulation were determined by histological analyses. The expression of several inflammatory and pro-fibrotic mediators were measured by quantitative RT-PCR.
Results: We found that bleomycin-induced dermal thickness and lipodystrophy were increased in MKP-1 deficient mice. Collagen accumulation in the dermis and mRNA expression of collagens 1A1 and 3A1 were enhanced in the skin from MKP-1 deficient mice as compared to the skin from WT animals. Affected skin from MKP-1 deficient mice presented increased expression of factors related to inflammation and fibrosis, namely IL-6, TGF-β1, fibronectin-1 and YKL-40 as well as chemokines MCP-1, MIP-1α and MIP-2.
Conclusions: This study demonstrates, for the first time, that MKP-1 deficient mice develop more severe bleomycin-induced dermal fibrosis than their WT counterparts, indicating that MKP-1 regulates the inflammatory and fibrotic processes typical for experimentally-induced scleroderma. These findings suggest that compounds which enhance expression/activity of MKP-1 have potential as novel drugs for the stage-specific modulation of the pathogenesis of scleroderma.
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SAT0322 ADAM-17 IS EXPRESSED IN THE INFLAMMATORY MYOPATHY, AND IS INVOLVED WITH INTERSTITIAL LUNG DISEASE (ILD)

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Background: A disintegrin and metalloprotesase (ADAM) family is protease that is thought to have an important role in tissue destruction and inflammatory reaction. ADAMs are also involved in the amputation from the cell surface of inflammatory cytokines. ADAM-17 is one of the ADAM family, and is first described as the protease responsible for tumor necrosis factor (TNF)-α shedding. The implication of ADAM-17 substrates in immunoregulation has made this enzyme an efficient therapeutic target in the treatment of a number of pathological conditions including airway inflammation and arthritis.
Objectives: The function of ADAM-17 in myositis is unclear. Therefore, we clarify the expression of ADAM-17 in inflammatory myopathy and the role of inflammation in interstitial lung diseases (ILD).
Methods: The serum were collected from the patients who were diagnosed with inflammatory myopathy in Showa University Hospital from 2003 to 2015. Twenty-six patients were diagnosed with polymyositis (PM), 34 patients were diagnosed with dermatomyositis (DM), and 10 patients were diagnosed clinically amyopathic dermatomyositis (CADM). Clinical manifestations and clinical data were also collected. The levels of ADAM-17 in the serum samples were measured using enzyme-linked immunosorbent assay (ELISA). ADAM-17 expression was determined in muscle tissues from DM using immunohistological staining. To determine that the role of lung fibrosis in inflammatory myopathy with ILD, we used human lung fibroblasts (HLF). ADAM-17 expression on HLF was also demonstrated by immunohistrogical staining. ADAM-17 expression in interleukine (IL)-6 and IL-6 receptor (IL-6R) stimulated HLF was performed by ELISA.
Results: ADAM-17 in inflammatory myopathy was significantly higher than in healthy control (n=19) (mean ± SEM; 1048±312 pg/ml and 36±18 pg/ml, respectively, $p<0.05$). ADAM-17 in corticosteroid and/or immunosuppressant treatment patient serum was also significantly decreased compared with in pre treatment patient serum (1465±562 pg/ml and 1059±503 pg/ml, respectively, $p<0.01$). In addition, ADAM-17 in inflammatory myopathy with ILD patients (n=46) was significantly higher than in non-ILD patients (n=24) (1379±454 pg/ml and 413±226 pg/ml, respectively, $p<0.05$), while ADAM-15 did not have the differences between ILD and non-ILD group. Finally, we found the expression of ADAM-17 in muscle biopsy tissue. Hence, ADAM-17 on HLF was expressed by immunohistochemistry. ADAM-17 in IL-6 and IL-6R stimulated HLF was significantly higher compared with non-stimulated HLF (48±6 pg/ml and 0±0 pg/ml, respectively, $p<0.05$).
Conclusions: ADAM-17 is expressed in inflammatory myopathies especially with ILD and expressed on HLF, suggesting that ADAM-17 may play the role in lung fibrosis. ADAM-17 may be a potential target in inflammatory myopathies with ILD.
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SAT0323 THE ENDOTHELIAL-TO-MESENCHYMAL TRANSITION (ENDOMT) IN SCLERODERMA CAN BE PREVENTED BY THE USE OF DUAL ENDOTHELIN RECEPTOR ANTAGONISTS BOSENTAN AND MACITENTAN

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Background: Systemic sclerosis (SSc) is characterized by early vascular abnormalities and subsequent fibroblast activation and differentiation into myofibroblasts, leading to fibrosis. Recently, endothelial-to-mesenchymal transition (EndoMT), a complex biological process in which endothelial cells lose their specific markers and acquire a mesenchymal or myofibroblastic phenotype, has been reported in SSc.
Objectives: The goal of the study was to evaluate the potential of endothelin-1 (ET-1) dual receptor antagonists bosentan (BOS) and macitentan (MAC) to antagonize EndoMT *in vitro*.
Methods: 20 patients with limited SSc were enrolled and underwent double skin biopsy (affected and nonaffected skin). Fibroblasts and microvascular endothelial cells (MVECs) were isolated from biopsies. Mono- or coculture of MVECs (isolated from nonaffected skin) with fibroblasts (isolated from affected skin and stimulated with ET-1 and transforming growth factor beta [TGF-β]) were performed. In cocultures, the MVEC layer was left undisturbed or was preincubated with either BOS or MAC. After 48 h of coculture, MVECs were analyzed for their capillary formation ability and for messenger RNA and protein expression of different vascular (CD31, vascular endothelial growth factor-A [VEGF-A], VEGF-A165b) and profibrotic (alpha-smooth muscle actin [α-SMA], collagen type I [Col I], TGF-β) molecules.
Results: MVECs showed a reduced capillary formation ability when cocultured with SSc fibroblasts with respect to mono cultures. CD31 and VEGF-A resulted in downregulation, while VEGF-A165b, the antiangiogenic isoform, resulted in upregulation. At the same time, mesenchymal markers α-SMA, Col I, and TGF-β resulted in overexpression in MVECs. Capillary formation ability was restored when MVECs were preincubated with BOS or MAC, also reducing the expression of mesenchymal markers and restoring CD31 expression as well as the imbalance between VEGF-A and VEGF-A165b.
Conclusions: BOS and MAC seem able to antagonize EndoMT phenomenon in MVECs *in vitro*. Blocking EndoMT is important for two reasons: first, because capillary formation ability in MVECs can be restored; second, because the endothelium-derived fibrotic development in SSc can be counteracted.
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SAT0324 INCREASED FREQUENCIES OF CIRCULATING CXCL10-, CXCL8- AND CCL4-PRODUCING MONOCYTES AND SIGLEC-3-EXPRESSING MYELOID DENDRITIC CELLS IN SYSTEMIC SCLEROSIS PATIENTS

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Background: Systemic sclerosis (SSc) is an inflammatory and fibrotic disease characterized by vascular dysfunction, excessive extracellular matrix deposition and immune dysregulation. Recent observations suggest that monocytes and dendritic cells (DCs) might be involved in SSc; including cell recruitment, trafficking, activation and an enhanced pro-fibrotic phenotype. Hence these cells might be important contributors to the disease pathogenesis. However, detailed analysis of circulating monocytes and DCs in SSc in relationship to disease activity has not been performed so far.
Objectives: To investigate the *ex vivo* pro-inflammatory properties of classical and non-classical monocytes as well as myeloid dendritic cells (mDCs) in SSc patients in relationship to disease activity.
Methods: This study enrolled 43 SSc patients, 30 classified as limited cutaneous SSc (lcSSc) and 13 as diffuse cutaneous (dcSSc). The healthy control group (HC) included 20 age- and gender- matched individuals. The Spontaneous production of CXCL10, CCL4, CXCL4 and IL-6 was intracellularly evaluated in classical and non-classical monocytes and Siglec-3-expressing mDCs from peripheral blood using flow cytometry. In addition, the production of these cytokines was determined upon toll like receptor 4 (TLR4) plus Interferon-γ (IFN-γ) *in vitro* stimulation.
Results: The frequency of non-classical monocytes spontaneously producing CXCL10 was increased in both lcSSc and dcSSC subsets of SSc patients ($p<0.05$) and CCL4 was augmented in the dcSSc patient subset ($p<0.05$). The proportion of CCL4 producing- mDCs were also elevated in dcSSc patients ($p<0.01$) and the percentage of mDCS producing CXCL10 only in lcSSc patients ($p<0.05$ compared to HC, but $p<0.01$ comparing to dcSSc). Upon *in vitro* stimulation the frequency of non-classical monocytes expressing CXCL8 was