Systemic sclerosis, myositis and related syndromes - aetiology, pathogenesis and animal models

AB0125
IL-25 PARTICIPATES IN KERATINOCYTE-DRIVEN DERMAL MATRIX TURNOVER AND IS REDUCED IN SYSTEMIC SCLEROSIS (SSC) EPIDERMIS
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Background: Evidence shows that dysfunctional SSc keratinocytes contribute to fibrosis by altering dermal homeostasis (1, 2). Whether interleukin-25 (IL-25), an IL-17 family member regulating many epidermal functions (3), takes part in skin fibrosis is unknown.

Objectives: To investigate the role of IL-25 in skin fibrosis.

Methods: The expression of IL-25 was evaluated by immunofluorescence and in situ hybridization in 10 SSC and 7 healthy donors (HD) skin biopsies. Epidermal equivalents (EE) reconstituted by primary HD keratinocytes primed with IL-25 was used to model to study transcriptomic changes induced by IL-25 in the epidermis. RNA expression profile in EE was characterized by RNAseq. The conditioned medium (CM) from primary SSC and HD keratinocytes primed with IL-25 was used to stimulate fibroblasts. IL-6, IL-8, MMP-1, type-I collagen (col-I), and fibronectin production by fibroblasts was assessed by ELISA.

Results: SSC epidermis expressed lower levels of IL-25 compared to HD. In EE, IL-25 regulated several molecular pathways related to wound healing and extracellular matrix (ECM) remodeling. Compared to control CM, the CM from IL-25-primed keratinocytes enhanced the fibroblast production of MMP-I, IL-8, IL-6, but not of col-I nor fibronectin. However, IL-25 significantly reduced the production of Col-I when applied directly to fibroblasts and partially inhibit γ-smooth muscle actin (γ-SMA) expression promoted by TGFβ. The activation of keratinocytes by IL-25 was receptor-dependent and evident after a very short incubation time (10min), largely mediated by IL-17, suggesting enhanced and specific release of preformed mediators.

Conclusion: These results show that IL-25 participates in skin homeostasis and its decreased expression in SSC may contribute to skin fibrosis by favoring ECM deposition over degradation.

REFERENCES:

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AB0127
BRONCHOALVEOLAR LAVAGE (BAL) FLUID AND SERUM FROM PATIENTS WITH SYSTEMIC SCLEROSIS WITH INTERSTITIAL LUNG DISEASE (SSC-ILD) PROMOTE A PRO-INFLAMMATORY GENE SIGNATURE IN HUMAN PRIMARY LUNG FIBROBLASTS
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Background: While circulating cytokines are frequently investigated in SSc patients, they may also play a role in local tissue, such as the lungs. Although most SSC patients show limited systemic inflammation, some studies have demonstrated that BAL fluid obtained from patients with SSC contains elevated levels of pro-inflammatory and pro-fibrotic cytokines. However, the relevance of the local milieu of the bronchial tree on the development of ILD has not been studied.

Objectives: To show the differential effect of BAL fluid and serum obtained from SSC patients with and without ILD on mRNA expression of pro-inflammatory and pro-fibrotic genes.

Methods: Serum and BAL fluid from 20 patients with SSC-ILD and 3 without ILD who were all treatment-naive. ILD diagnosis was based on HRCT and lung function tests. Normal human primary lung fibroblasts were cultured and treated with either BAL fluid (5%) or serum (0.5%) from all individual patients. No treatment (CTRL) or treatment with pooled serum from healthy controls were used as control. After 4h, fibroblasts were harvested in TRIzol. The mRNA expression levels of inflammatory markers (interleukin-6, -8, -10, IP-10, fibroblast growth factor 10, tumor necrosis factor), pro-fibrotic markers (procollagen-1, -3, -5) and pro-fibrotic markers (Connective Tissue Growth Factor, CTGF, Transforming Growth Factor, TGF-β, Alpha- Smooth Muscle Actin, γ-SMA) were assessed using RT-qPCR.

Results: Fibroblasts treated with either SSC or SSC-ILD BAL fluids showed a significantly higher mRNA expression of IL-6, IL-8, and IL-10 compared to controls (Figure 1a).

The same was observed for IP-10, except for SSC serum which was not significant (Figure 1b). When comparing the effects of BAL fluids between SSC or SSC-ILD patients, the effect of SSC-ILD BAL fluid was strikingly more profound.

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than observed with SSC on both IL-6 and IP-10 (Figure 1 a,b). Similar effects were seen when fibroblasts were treated with SSc serum, where serum from SSC-ILD resulted in significantly higher expression of IL-6 and IP-10 compared to SSC (Figure 1 a,b). The effect of serum and BAL on IL-6 gene expression were strongly and significantly correlated (r=0.9; P=0.015) while were weakly correlated regarding IP-10 expression (r=0.4; P=0.3) (not shown). The fibroptic markers TGF-β and α-SMA showed no difference in expression in BAL or serum-treated fibroblasts compared to controls (Figure 1 c,d). Only the fibroblasts treated with SSc-ILD serum showed a significant increase in mRNA expression of the early fibrosis marker CTGF when compared to control serum (Figure 1 e).

**Conclusion:** We showed a clear pro-inflammatory effect of BAL fluid obtained from patients with SSC on human fibroblasts as demonstrated by mRNA expression of IL-6 and IP-10. This pro-inflammatory effect was 5-10 times more profoundly observed in SSc patients with ILD compared to those without ILD. Similar effects were observed when fibroblasts were treated with serum obtained from the same SSc patients where the BAL fluid and serum of each patient seemed to provoke a concordant pro-inflammatory effect. Although further studies are warranted, our results underline the systemic nature of SSc and provide new insights into the role of and interaction between the local bronchial and systemic milieu in ILD development.

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**AB0129**

**MYOSITIS-SPECIFIC AUTOANTIBODIES IN CLINICAL PRACTICE: IMPROVING THE PERFORMANCES OF THE IMMUNODOT**

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**Background:** Idiopathic inflammatory myopathies (IIM) or myositis are a group of rare autoimmune diseases that combine muscle weakness and multi-visceral damage. The discovery of IIM-specific autoantibodies (aAbs) and their associations with clinical phenotypes has improved diagnostic and classification criteria. Faced with the large number of these aAbs, multiplexed techniques have emerged. Among them, the immunodot is simple, rapid, and inexpensive, but has been several times criticized for its lack of specificity.

**Objectives:** Our objective was to evaluate the current interpretation criteria of the D-Tek immunodot and to propose new interpretation rules based on clinical criteria in order to improve its reliability.

**Methods:** We included in this retrospective study patients tested positive result on the semi-quantitative myositis/synthetase immunodots at manufacturer's threshold (≤5 UA), for at least one of the 15 aAbs; anti-SRP, anti-NXP2, anti-TIF1, anti-SAE (1 and 2), anti-Mi2, anti-MDA5, anti-Jo1, anti-PL7, anti-PL12, anti-EJ, anti-OJ, anti-KS, anti-ZO, anti-HA. Specificity of the immunodots was further evaluated using 60 healthy and anonymous subjects (French blood bank, Toulouse, France). The clinical diagnosis and sub-classification retained by the clinician in charge of the patient was used as a reference for attribution to the different diagnostic categories: myositis group, non-mysitis group, subgroups. For the myositis group, 7 specific aAbs were considered; immune-mediated necrotizing myopathy (n=4); dermatomyositis (n=6); anti-synthetase syndrome (n=36); inclusion body myositis (n=11); overlap myositis with another connective tissue disease (n=7); polymyositis (n=8); and unclassified myositis (n=6). For the non-mysitis group, patients were subdivided in 4 subgroups: autoimmune or inflammatory diseases (n=72); isolated and diffuse interstitial lung disease (n=28) not associated with other myositis criteria; other non-inflammatory myopathies (n=8) including genetic, metabolic, and toxic myopathies; and other diseases (n=36). The immunodot interpretation thresholds were evaluated both in relation to the manufacturer’s threshold, and by considering the phenotypes and clinical diagnoses using a ROC method (Youden’s index).

**Results:** Among 270 patients included between 01/07/2016 and 30/06/2020, 129 patients were classified as myositis (median age 58 years, 60% women, 52% DM and 28% AS) and 142 (53%) in non-mysitis. Among the 15 aAbs analyzed, none were detected in the healthy control group but they were represented in both myositis and non-mysitis group. Among them only 2 (anti-Jo1, anti-Mi2) predominated in the myositis group, and 1 (anti-TIF1) in the non-mysitis group (Fisher’s test). As quantitative values were found different for 6 aAbs (Mann Whitney test), a clinical threshold was calculated to discriminate myositis from non-mysitis groups (ROC curve) allowing to determine an odds ratio (OR). Accordingly, 4/15 (%) aAbs were found associated with myositis: anti-SRP (at 28UA: OR=3.24 95% CI [1.01-10.46], p=0.048), anti-MDA5 (at 15UA: OR=4.36; 95% CI [1.19-15.99], p=0.048), anti-Mi2 (at 28UA: OR =3.24; 95% CI [1.01-10.46], p=0.026), anti-Jo1 (at 15UA: OR= 12.20; 95% CI [2.78-53.52], p<0.0001). All positive predictive values were improved by using a clinical threshold although some of them did not reach significance due to their infrequency.

**Conclusion:** In this retrospective work, despite missing data, the clinical phenotypes of myositis patients and their distribution according to aAbs were identified by qPCR. Data is normalized to housekeeping gene 18S for normalization.