POS0431

FIBROBLASTS ARE NOT JUST FIBROBLASTS - CLEAR DIFFERENCES IN ECM PRODUCTION BETWEEN DERMAL AND LUNG FIBROBLASTS IN RESPONSE TO GROWTH FACTORS

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Background: Many systemic sclerosis (SSc) patients develop lung fibrosis, which contribute significantly to increased mortality¹. Activated and proliferating fibroblasts are responsible for the excessive extracellular matrix (ECM) formation and stiffening of the connective tissue leading to skin and lung fibrosis. There is currently no effective treatment for the fibrosis in SSc and there is therefore a medical need for further understanding the pathogenesis of fibrosis. Fibrosis is associated with different growth factors, including tumor growth factor beta 1 (TGF- β 1) and platelet derived growth factor-ab (PDGF-ab)².

Objectives: We investigated how stimulation with TGF- β 1 and PDGF-ab affected the migration capacity and the ECM production using translational biomarkers of type I, III and VI collagens in healthy human dermal and lung fibroblasts.

Methods: The fibroblasts were grown in DMEM media containing 0.4% fetal calf serum, ficoll (to produce a crowded environment) and ascorbic acid for up to 12 days. The cells were stimulated with TGF- β 1 [0.04-1 nM] or PDGF-ab [3 nM] at treatment initiation and changed twice a week. Non-stimulated fibroblasts (w/o) were used as control. A wound was induced by scratching the cells at day 1 after treatment initiation and the migration was followed for 2 days. Type I, III and VI collagen formation (PRO-C1, PRO-C3 and PRO-C6, respectively) were evaluated by validated ELISAs (Nordic Bioscience) in supernatant from day 0, 4, 8 and 12. Statistical analysis included 2-way ANOVA and Dunnett's test.

Results: The PDGF-ab stimulated dermal fibroblasts migrated significantly more than the non-stimulated ($p < 0.0001$) and TGF- β 1 stimulated ($p < 0.001$) dermal fibroblasts 48 hours after the scratch (migration app. 70%, 30% and 30% respectively). There was no difference between the migration of the non-stimulated, TGF- β 1 and PDGF-ab stimulated lung fibroblasts after 48 hours, as all migrated to approximately 70%.

TGF- β 1 stimulation led to a significant increase in type I collagen formation (PRO-C1) in both dermal and lung fibroblasts from day 4 and onwards compared to w/o ($p < 0.0001$). TGF- β 1 also led to a significant increase in type III collagen formation (PRO-C3) from day 8 in lung fibroblasts compared to w/o ($p < 0.0001$). PDGF-ab stimulation led to a significant increase in type III collagen formation in dermal fibroblasts from day 8 compared to w/o ($p < 0.0001$). PDGF-ab stimulation led to a significant increase in type VI collagen formation (PRO-C6) in both dermal and lung fibroblasts from day 4 and onwards compared to w/o ($p < 0.0001$).

Conclusion: PDGF-ab increased the migration activity of the dermal fibroblasts, where the lung fibroblasts had a general high migration activity. The dermal and lung fibroblasts showcase the same ECM production within both type I and type VI collagen formation. The two fibroblasts types did however react opposite each other regarding the type III collagen formation: the dermal fibroblasts responded to PDGF-ab stimulation, where the lung fibroblasts responded to the TGF- β 1 stimulation. The clear differences in the ECM production between the dermal and lung fibroblasts can be important in the search for an effective treatment for fibrosis in SSc and related lung fibrosis.

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Figure 1. TGF- β 1 and PDGF-ab can stimulate ECM production in both dermal and lung fibroblasts. Synthesis of type I (a), type III (b), and type VI (c) collagen in dermal fibroblasts and type I (d), type III (e), and type VI (f) collagen in lung fibroblasts. Data are shown as mean $\pm$ SD of 3 separate experiments with 4 replicates each. Data were analyzed by two-way ANOVA with Dunnett's multiple comparisons test against the control (w/o).

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POS0432

POST-IMMUNIZATION WITH COLLAGEN V CONFERS A DISADVANTAGEOUS IMMUNE MATRICES MICROENVIRONMENT FOR EARLY LUNG FIBROSIS IN A SSc MOUSE MODEL

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Background: The autoimmunity and inflammation phenomena observed in systemic sclerosis (SSc) lead to the dysregulation of type V collagen (Col V) and extracellular matrix (ECM) deposition of Col V, collagen I (Col I) and collagen III (Col III) fibers, characteristic of pulmonary fibroproliferative disease. T cells are the predominant inflammatory infiltrate in the lungs and are believed to produce cytokines that drive the synthesis of matrix proteins by fibroblasts, resulting in excessive fibrosis. Although most studies to date have focused on CD4+ T and CD20+ B immune cells in some SSc subsets, cytotoxic CD8+ T cells are also involved in the pathogenesis of SSc. We previously reported that reactivity to the ColV-self antigen predicts late SSc lung fibrosis in mice immunized with Col V after 120 days; however, the profile of underlying immune matrices in early SSc lung fibrosis remains unclear.

Objectives: To quantify T and B-cells and collagen fibers in the days following immunization with Col V in a mouse model mimicking human lung involvement in SSc.

Methods: Female C57BL/6 mice (n=18) were immunized subcutaneously with human Col V (150 μ g) in complete Freund's adjuvant, followed by two intramuscular boosters (IM-COLV). The control group (n=18) did not receive Col V. Three groups of animals (n=6 each) were euthanized on days 15, 30 and 45 after immunization. The lung sections were fixed in 10% buffered formalin, embedded in paraffin, and sectioned at 4 μ m. Immunohistochemistry, immunofluorescence and histomorphometry were performed to phenotype and quantify CD4 and CD8 T-cells, CD20 B-cells, and Col I, III and V, respectively.

Results: We found that T-cells (CD4⁺), cytotoxic T cells (CD8⁺), and B-cells (CD20⁺) were present in high densities in the lungs of IM-COLV mice, with variation in the absolute abundance of each of these immune cell lineages at 15, 30 and 45 days after immunization with Col V. In fact, a significantly higher density of CD4⁺, CD8⁺, and CD20⁺ was detected early along the connective matrix of alveolar septa at 15 days after Col V immunization. In addition, higher density of Col I, III and V fibers were also observed early (Table 1). Although early SSc-lung fibrosis is generally considered to be linked to inflammatory cytokines, we also infer that cytotoxic T-cells may induce alveolar cells apoptosis by triggering kinases on macrophages and then accelerate fibrotic scar.

Conclusion: Our study quantitatively establishes that the densities of CD4+ CD8+ and CD20+, as well as Col I, Col III and Col V can be characterized as markers of early lung fibrosis in SSc. These findings suggest that cytotoxic T cells may induce apoptosis and secrete profibrotic cytokines to induce the deposition of collagen fibers in the lungs, inferring that early B cell depletion may improve early lung fibrosis in SSc.

	Post-Col V Immunization (Days)					
	15		30		45	
	Control	IM-COLV	Control	IM-COLV	Control	IM-COLV
Immune Cells						
CD4+ cells/mm ² (%)	13.85 $\pm$ 0.46	34.14 $\pm$ 1.55*	12.63 $\pm$ 0.56	23.38 $\pm$ 0.83	14.32 $\pm$ 0.52	23.62 $\pm$ 0.40
CD8+ cells/mm ² (%)	13.84 $\pm$ 0.60	39.40 $\pm$ 0.99**	12.42 $\pm$ 0.52	22.87 $\pm$ 0.85	12.93 $\pm$ 0.57	23.07 $\pm$ 1.00
CD20+ cells/mm ² (%)	12.12 $\pm$ 1.01	33.84 $\pm$ 1.43#	11.70 $\pm$ 1.14	15.23 $\pm$ 6.70	12.46 $\pm$ 0.50	28.24 $\pm$ 0.67
Collagen Fibers						
Col V (area fraction)	0.05 $\pm$ 0.003	0.12 $\pm$ 0.01	0.08 $\pm$ 0.003	0.14 $\pm$ 0.01††	0.07 $\pm$ 0.003	0.11 $\pm$ 0.003
Col I (area fraction)	0.16 $\pm$ 0.01	0.38 $\pm$ 0.05#	0.14 $\pm$ 0.01	0.25 $\pm$ 0.03	0.15 $\pm$ 0.02	0.24 $\pm$ 0.01
Col III (area fraction)	0.16 $\pm$ 0.003	0.28 $\pm$ 0.01†	0.14 $\pm$ 0.004	0.25 $\pm$ 0.05	0.13 $\pm$ 0.007	0.16 $\pm$ 0.02
Total Col μ g/mg	2.7 $\pm$ 0.29	3.99 $\pm$ 0.58	3.15 $\pm$ 0.19	3.77 $\pm$ 0.27	2.87 $\pm$ 0.30	4.99 $\pm$ 0.60

Data are shown as mean $\pm$ SD. Comparisons were done using ANOVA/Dunn's Multiple comparison test ($P < 0.05$). *15 vs 30 and 45 days; **15 vs 30 and 45 days; #15 vs 30 and 45 days; †15 vs 45 days; ††30 vs 45 days. Col I, collagen type I; Col III, collagen type III; Col V, collagen type V.