5. Decision making in the T-cell immune system

A98 TUMOUR NECROSIS FACTOR α RECEPTOR ACTIVATED T CELLS OF PATIENTS WITH SYSTEMIC SCLEROSIS ARE DEFICIENT IN IL-10 EXPRESSION BUT PROMOTE MYOFIBROBLAST DIFFERENTIATION VIA IL-6 AND TGF- β

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Background and objectives Systemic sclerosis (SSc) is an auto-inflammatory multisystem disease leading to fibrosis of the skin and inner organs. T lymphocytes are key effector cells in the affected tissue. The role of lymphocytes in the transition from inflammation to fibrosis is unclear. Previous data suggested a possible pro-fibrotic effect of tumour necrosis factor-receptor (TNF-R) 2.

Materials and methods Skin punch biopsies from affected skin of 12 SSc patients (six limited, six diffuse) were digested in dispase and collagenase and processed for multicolour flow cytometry (CD45, HLA-DR, auto-fluorescence, CD14, CD1a, C3, CD4, CD8, TNF-R1 and 2). α -Smooth muscle actin (α -SMA) positive myofibroblasts and CD3⁺ lymphocytes were furthermore quantified in skin biopsies of 26 patients of a control SSc patient cohort by immunohistochemistry. For functional analyses, T lymphocytes were isolated from peripheral blood of 12 patients using CD3 selection and activation via $\pm$ CD3/CD28 beads. TNF-R were stimulated with selective CysTNF mutants at concentrations of 100 ng/ml. Real-time PCR and multiplex cytokine assay was performed for cytokine expression. Fibroblasts of SSc patients were incubated in conditioned (TNF-R stimulated) lymphocyte medium; α -SMA was detected by Western blot.

Results Higher numbers of lymphocytes per CD45 cells (45.7% vs 26.6%), but less CD1a⁺ (1.0% vs 4.1%) cells were detected in the dermis of patients with diffuse SSc compared to limited SSc. Lymphocyte infiltration correlated with skin thickening (modified Rodnan skin score, mRSS) of the patients ($p=0.004$) and myofibroblast infiltration ($p<0.05$). TNF-R2 was significantly up-regulated on CD3, CD4 and CD8 subsets in patients with diffuse but not limited disease. TNF-R1 was not significantly upregulated on dermal lymphocytes. The mean intensity fluorescence of TNF-R2 but not 1 correlated with the mRSS ($p=0.01$). Activation of SSc lymphocytes selective TNF-R agonists showed that TNF-R1 stimulation resulted in a significantly higher interleukin (IL)-6 expression

whereas TNF-R2 stimulation lead to a higher TGF- β expression, compared to healthy individuals. Conversely, CD3/CD28 activated lymphocytes of SSc patients expressed significantly less IL-10 upon TNF-R2 stimulation compared to lymphocytes from healthy individuals. When the authors incubated control fibroblasts in conditioned medium, medium from healthy TNF-activated lymphocytes *inhibited* α -SMA expression. In contrast, conditioned medium from TNF-activated SSc lymphocytes *increased* α -SMA expression. In dermis of a SSc patient who underwent lymphoablative treatment, the authors observed a reduced number of myofibroblasts and lymphocytes with a reversibility of TNF-R2 expression.

Conclusion Lymphocyte infiltration and TNF-R expression play a role in SSc via enhanced IL-6, TGF- β and myofibroblast differentiation. Deficient IL-10 expression might impair the resolution of inflammation in SSc.