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Autoantibodies in serum of systemic scleroderma patients: peptide-based epitope mapping indicates increased binding to cytoplasmic domains of CXCR3

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Introduction: Systemic sclerosis (SSc) is a severe chronic autoimmune disease with high morbidity and mortality. Sera of patients with SSc contain a large variety of autoantibody (aab) reactivities. Among these are functionally active aab that bind to G protein-coupled receptors (GPCR) such as C-X-C motif chemokine receptor 3 (CXCR3) and 4 (CXCR4). Aab binding to the N-terminal portion of these two GPCRs have been shown to be associated with slower disease progression in SSc, especially deterioration of lung function.

Methods: We used peptide ELISA with serum samples in combination with a hierarchical Bayesian model for mapping of epitopes on CXCR3. For in silico prediction of epitopes on CXCR3, we used the ABCpred online tool.

Results: To identify linear epitopes of anti-CXCR3 aabs, we used a peptide array of 36 overlapping 18-20mer peptides covering the entire CXCR3 sequence for ELISA to compare the binding pattern of SSc patient sera (N=32) and healthy controls (N=30). We found increased binding of SSc patient sera to intracellular epitopes within CXCR3, while the binding signal to extracellular portions of CXCR3 was found to be reduced in Ssc patients. Experimentally determined epitopes showed a good correspondence to those predicted by the ABCpred tool. To verify these results and to translate them into a novel diagnostic ELISA, we combined the peptides that represent SSc-associated epitopes into a single ELISA and evaluated its potential to discriminate SSc patients (N=31) from normal healthy controls (N=47). This ELISA had a sensitivity of 0.61 and a specificity of 0.85.

Conclusions: The clinical relevance of anti-CXCR3 aabs may crucially depend on epitope specificity, with SSc sera preferentially binding to intracellular epitopes, while healthy control sera appear to recognize an extracellular epitope in the N-terminal domain. Moreover, we propose an ELISA that could detect intracellularly binding anti-CXCR3 aabs for the monitoring of Ssc patients.