Characterization of the anti-centromere antibody response in Systemic Sclerosis patients suggests a broad and active B cell response

Introduction
Systemic Sclerosis (SSc) is a rare, heterogeneous autoimmune disease characterized by microvascular damage, organ fibrosis and immune dysfunction. Autoantibodies are detected in >95% of patients, the most prevalent being anti-centromere (ACA) and anti-topoisomerase (ATA) antibodies. Although used for diagnosis, little is known about the underlying auto-reactive B cell responses. In particular, the ACA B cell response has been poorly studied.

Objectives
Characterization of the ACA B cell response in SSc patients.

Methods
ACA IgG, IgA and IgM levels were measured in serum samples of 167 ACA IgG+ SSc patients. Patients were divided in a SSc (fulfilling ACR 2013 criteria, n=132) and a very early SSc group (fulfilling VEDOSS criteria, n=35). Additionally, PBMCs from ACA IgG+ SSc patients (and ATA IgG + SSc and healthy donors (HD) as control) were cultured either in the presence of CD40L expressing fibroblasts, IL-21 and BAFF or without stimulation. Levels of ACA IgG, IgA and IgM (and total Ig) were measured after one week of culture using ELISA.

Results
ACA IgG+ SSc patients displayed a broad isotype usage with 75% being ACA IgA+ and 68% being ACA IgM+ in serum. Patients within the SSc group showed higher ACA IgG levels and a higher percentage of ACA IgM positivity compared to the very early SSc group. ACA IgG, IgA and IgM could be measured in ACA SSc PBMC culture medium following stimulation, but not in ATA SSc and HD, indicating the presence of circulating ACA B cells of all three isotypes. In cultures that yielded sufficient Ig production, ACA IgG was detectable in 7/9 ACA SSc patients, ACA IgA in 3/7 and ACA IgM in 2/7. Furthermore, ACA IgG production was also detected in the absence of stimulation in 5/9 patients, suggesting the presence of ACA-producing plasmablasts in the circulation. No spontaneous production of anti-Tetanus Toxoid antibodies, a control recall response, was observed.

Conclusions
ACA+ SSc patients display a broad range of isotype usage in their ACA response, reflected both by ACA serum levels and presence of ACA IgG-, IgA- and ACA IgM-producing B cells in the peripheral blood. Additionally, ACA IgG production by unstimulated PBMCs points towards continuous differentiation of memory cells into antibody secreting cells. These data, together with differential isotype profiles between very early SSc and SSc patients, provide insight into the ACA B cell response and its potential involvement in disease-relevant pathogenetic processes.

Disclosure of Interest
None declared.