cultured B cells improves their regulatory and or pro-inflammatory properties.

Materials and Methods Serum from SLE patients, RA patients (disease control) and normal controls was analysed for sema3A levels and was correlated with clinical parameters of SLE. The expression of sema3A on Bregs was compared between SLE patients and normal individuals. In addition to this, the expression of CD72 and TGF-β (inhibitory molecules), and TLR-9 in B cells were assessed in both groups following the addition of recombinant sema3A to cultured B cells.

Results 1. Serum sema3A levels were significantly lower in SLE patients comparing to that of RA patients and much lower than in normal controls (55.04 ± 16.30 ng/ml versus 65.54 ± 14.82 ng/ml, versus 74.41 ± 17.60 ng/ml, respectively, $P < 0.0001$). 2. Sema3A levels were inversely correlated with SLE disease severity, kidney involvement and anti-cardiolipin-ab. 3. Sema3A expression on Bregs was significantly lower in SLE patients comparing normal individuals (52.2 ± 5.8% versus 82.6 ± 6.4%, $P < 0.0001$, respectively). 4. The expression of both CD72 and TGF-β was significantly decreased (37.88%, 8.6% respectively) in Bregs of SLE patients versus that on normal Bregs (49.26%, 14.74%, respectively; $P = 0.001$). However, following the addition of sema3A to cultured B cells, a significant increase of TGF- β and CD72 was noticed (altered in SLE patients, when compared to that of normal individuals). 5. The addition of sema3A to CpG-ODN stimulated B cells from SLE patients reduced TLR-9 expression by almost 50%.

Conclusions 1. This is the first study where sema3A is shown to be altered in both serum and on Breg cells of SLE patients. 2. Sema3A enhances the regulatory properties of Bregs, but this effect is shown to be altered in SLE. 3. Sema3A should be considered a future therapeutic tool in SLE.

A5.29 SPONTANEOUS PRODUCTION OF ANTI-CITRULLINATED PROTEIN ANTIBODIES IN CULTURES OF PERIPHERAL BLOOD MONONUCLEAR CELLS AND SYNOVIAL FLUID MONONUCLEAR CELLS ISOLATED FROM PATIENTS WITH RHEUMATOID ARTHRITIS

doi:10.1136/annrheumdis-2013-203219.29

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Background and Objectives Anti-citrullinated protein antibodies (ACPA) are among the most important molecular candidates that could drive the inflammatory immune response in a subset of patients with rheumatoid arthritis (RA). So far, however, little is known on the phenotype and functional characteristics of ACPA producing B cells. Therefore, we studied ACPA producing B cells using ex-vivo cultures of peripheral blood mononuclear cells (PBMC) and synovial fluid mononuclear cells (SFMC).

Materials and Methods PBMC as well as SFMC from patients with ACPA-positive RA were cultured in 96 well plates without the addition of exogenous stimuli. Cultures were maintained for several weeks, with weekly complete replacement of the culture medium. Every week, supernatants were assessed for the presence of ACPA-IgG and total IgG by ELISA. B cell subsets within the culture populations were determined by flow cytometry at several time points.

Results Circulating, spontaneously ACPA producing B cells were readily detectable in peripheral blood of ACPA positive RA patients, but not in ACPA negative RA patients or healthy donors. FACS sorting experiments comparing isolated B cell subsets located spontaneous ACPA production to the plasmablast compartment. Memory B cells were capable of ACPA production upon stimulation. In some culture wells, ACPA production was stable and detectable for up to 3 months. In a similar manner, we observed spontaneous, long-lasting ACPA production in (paired) SFMC cultures. The latter showed an up to 200 fold increase in ex vivo ACPA production compared to PBMC, but only a minor increase in the secretion of non-specific IgG. B cell numbers in PBMC and SFMC were comparable in the starting population.

Conclusions ACPA specific plasmablasts circulate in the peripheral blood of patients with ACPA positive RA. Upon isolation, peripheral blood B cells can secrete ACPA spontaneously for several months. This observation suggests that ACPA specific B cells are either continuously recruited from the memory compartment, or that a subset of ACPA specific plasmacells might have the capacity to survive for extended periods of time. In SFMC, the frequency of ACPA specific B cells is strongly increased. These observations point to a continuously active, ACPA specific immune response in RA.

A5.30 SYSTEMIC INFLAMMATION AND B-CELLS IN RHEUMATOID ARTHRITIS

doi:10.1136/annrheumdis-2013-203219.30

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Background and Objectives Rheumatoid arthritis (RA) is heterogeneous in clinical symptoms, clinical parameters, pathogenesis and gene expression levels. Previously, we demonstrated variation in B-cell related gene expression between RA patients. The aim was to explore the relation of B-cell related gene expression to clinical parameters of disease severity in early arthritis (EA) and established RA (esRA).

Methods B-cell related gene expression (B-cell score) was determined in peripheral blood cells of 26 EA and 180 esRA patients, using multiplex real-time PCR. For the EA cohort, B-cell counts were also measured using flow cytometry. The esRA cohort was (randomly) divided into test and validation group of each 90 RA patients, with a mean DAS28 of 5.0 and 5.2, respectively. Associations were assessed between B-cell scores and the clinical disease parameters DAS28, CRP, RF, anti-CCP, nodules and erosions in all cohorts and B-cell counts only in EA cohort. Statistical testing was executed according to a bootstrap method which randomises the esRA group a 1000 times into two equally sized groups.

Results We demonstrated that the B-cell score reflected the peripheral blood B-cell count ($p < 0.0001$, $r = 0.7463$). In EA, the B-cell score revealed a significant negative correlation with CRP levels ($p = 0.0175$; $r = –0.4618$). In the esRA group we also observed a negative correlation between the B-cell score and CRP levels ($p = 0.0006$, $r = –0.3542$; $p = 0.0096$ after Benjamini-Hochberg multiple testing correction). This result was confirmed in the independent validation group ($p = 0.0356$; $r = –0.2218$). Additionally, we performed a randomisation with the bootstrap method, which showed the same significant correlations in almost all cases. However, no correlations were found between B-cell score and DAS28, RF, anti-CCP, nodules or erosions.

Conclusions The B-cell score reflects the B-cell count in RA and a low B-cell count is associated with an increased marker of systemic inflammation in RA.

A5.31 THE ROLE OF BOB1 IN RHEUMNATOID ARTHRITIS: POTENTIAL IMPLICATIONS FOR AUTOIMMUNITY

doi:10.1136/annrheumdis-2013-203219.31

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