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Disclosure of Interest None declared.

P006

INVESTIGATION OF IGA IMMUNE COMPLEX CAPTURE BY FCRL4+ B CELLS IN PERIPHERAL BLOOD AND SYNOVIAL FLUID

¹J Cameron*, ¹E Clay, ¹A Filer, ²G Vidal-Pedrola, ²A Anderson, ²J Isaacs, ¹K Raza, ¹D Scheel-Toellner. ¹Rheumatology Research Group, University of Birmingham, Birmingham; ²Institute of Cellular Medicine, Newcastle University, Newcastle, UK

10.1136/annrheumdis-2018-EWRR2019.3

Career situation of first and presenting author Student for a master or a PhD.

Introduction Increasing evidence points to a mucosal origin of the autoimmune process in the development of rheumatoid arthritis. A B cell subset identified by surface expression of Fc receptor-like 4 (FcRL4) associates with inflammation in the mucosa associated lymphoid tissue.¹ Our group identified these cells in the synovium of RA patients.² They express high levels of RANKL and participate in the autoimmune response to citrullinated proteins.^{3–4} Recent *in vitro* work suggested that FcRL4 may be a low-affinity receptor for aggregated IgA.⁵ FcRL4 +B cells in RA SF express low levels of CD21 and high levels of CD11c, a phenotype they share with Age-Associated B cells (ABC). Very low numbers of ABC are found in blood of healthy individuals but their frequency increases with age however their exact role in RA is not yet understood.⁶

Objectives 1) Investigate the binding activity of IgA immune complexes to FcRL4⁺ B cells in tonsil, RA synovial fluid, and the peripheral blood of healthy individuals and RA patients. 2) Determine if FcRL4 can specifically capture IgA from the RA SF. 3) Examine the specificity of FcRL4 for secreted and serum IgA and for different IgA isotypes.

Methods SF, peripheral blood and tonsil mononuclear cells were labelled for FcRL4, IgA, IgG, and CD19 (PBMCs were also labelled for CD21 and CD11c) and analysed by flow cytometry. Cells pre-treated with a pH3 buffer to remove receptor-bound immunoglobulins were compared with untreated cells to distinguish IgA B cell receptor expression from receptor-bound IgA Immune complexes. An FcRL4 expressing cell line was incubated with RA synovial fluid and purified human IgA to assess FcRL4s specificity.

Results Receptor-bound IgA Immune complexes were observed on *ex vivo* RA SF and tonsil FcRL4⁺ B cells but not on FcRL4⁺ B cells (p=0.001). Intriguingly, this IgA capture was not observed in peripheral blood FcRL4 +ABC from HC or RA patients. Furthermore, the proportion of B cells with IgA BCRs was increased in tonsil and SF FcRL4 +B cells but not in peripheral FcRL4⁺ B cells, compared to FcRL4⁺ B cells. Using a cell line, we show that FcRL4 specifically binds IgA immune complexes from SF and shows a preference for IgA1 over IgA2 (p=0.02).

Conclusions FcRL4 is a receptor for IgA immune complexes in RA joints. RA SF and tonsil FcRL4 +B cells RA SF differ from peripheral blood FcRL4 +ABC in their *in vivo* capture of IgA immune complexes and in their frequency of IgA BCRs, suggesting distinct function and origin of these cells.

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Disclosure of Interest None declared.

P007

AUTOANTIBODIES' TITRE CHANGES DURING ANTI-BLYS TREATMENT IN SYSTEMIC LUPUS ERYTHEMATOSUS

¹I Cavazzana*, ¹C Pozzari, ¹R Kumar, ²R Ottaviani, ¹M Fredi, ²S Piantoni, ²A Tincani, ²F Franceschini. ¹Rheumatology Unit, Spedali Civili, Brescia, Italy; ²Clinical and Experimental Science, University of Brescia, Rheumatology Unit, Spedali Civili, Brescia, Italy

10.1136/annrheumdis-2018-EWRR2019.4

Career situation of first and presenting author Assistant.

Introduction Belimumab, a human monoclonal antibody anti-soluble B lymphocytes stimulator, is the only biological drug approved for Systemic Lupus Erythematosus (SLE). It reduces disease activity and blocks damage progression.^{1–2} Anyway, no definitive results have been published regarding its effect on autoantibodies' titre.

Objectives To analyse the modulation of anti-dsDNA, anti-ENA and anti-phospholipid antibodies' titre by Belimumab in patients affected by SLE.

Methods 50 SLE³ patients starting Belimumab were enrolled. Sera were collected at baseline (T0) and every 6 months until 24th month. Clinical features and disease activity index (namely SLEDAI-2K)⁴ were collected at any timepoints. Anti-dsDNA were analysed by radioimmunological method, anti-cardiolipin (aCL) and anti-β2 glycoprotein I (anti-β2GPI) were detected by home-made ELISA.^{4–5} Anti-ENA were detected by ELISA; positive sera were further characterized by ELISA to identify anti-Ro, anti-La, anti-ribosomal P protein (ribP), anti-Sm and anti-U1RNP specificities.

Results At T0 anti-dsDNA were positive in 86%, anti-ENA in 50%, a CL in 20% and antiβ2GPI in 28% of cases, respectively. A negative seroconversion was detected in 9/43 anti-dsDNA (21%); in 5/12 anti-beta2GPI IgG (40%) and in 3/10 aCL IgG (30%) sera, mostly at T6. Among anti-ENA specificities 6/10 (60%) anti-ribP and 3/17 (17,6%) anti-Sm positive sera became negative; while anti-Ro and anti-U1RNP antibodies resulted stable during treatment. A significant decrease of anti-dsDNA titre was observed at T6 (p 0,0011); T12 (p: 0,014); T18 (p: 0,0031) and T24 (p: 0,013). Anti-beta2GPI IgM isotype showed significant reduction at T6 (p: 0,0032), T12 (0,001), T18 (p: 0,002) and T24 (p: 0,011). ACL antibodies showed significant decrease only for IgG isotype at T18 (p: 0,001). Anti-ribP antibodies showed a significant titre decrease at T6 (p: 0,015) and T12 (p:0,0078), with complete negative seroconversion at T18. Anti-Sm antibodies significantly dropped down at T6 (p:0.032), than maintained a stable titre during time. A significant correlation between anti-dsDNA titre anti-ribP titre and between SLEDAI ratio (SLEDAI value/SLEDAI T0) and anti-ribP ratio (value/value T0) were found.

Conclusions Belimumab treatment induced a significant reduction of high-affinity anti-dsDNA, anti-Sm and anti-ribP antibodies titre. The decrease of anti-ribP titre correlates with anti-dsDNA titre and disease activity.

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Disclosure of Interest None declared.

P008

INFLUENCE OF M-CSF POLARISATION OF MONOCYTES AND ACTIVATION OF THE M-CSF MACROPHAGES BY PRO-INFLAMMATORY OR OTHER POLARISING CYTOKINES, ON EXPRESSION OF PEPTIDYLARGININE DEIMINASES 2 AND 4

M-C Méchin, G Serre, C Clavel*. *Unité Différenciation Epithéliale et AutoImmunité Rhumatoïde, UDEAR, Toulouse Cedex 9, France*

10.1136/annrheumdis-2018-EWRR2019.5

Career situation of first and presenting author Post-doctoral fellow.

Introduction Autoantibodies to citrullinated proteins (ACPA) are specifically associated to rheumatoid arthritis (RA) and likely involved in its pathophysiology. ACPA are produced in the inflamed synovial tissue (ST) where peptidylarginine deiminase (PAD) 2 and 4, responsible for fibrin citrullination, generate the ACPA-targeted epitopes. PAD2 and 4 are expressed in the intima and the subintimal inflammatory infiltrates by CD68⁺ mononuclear cells.¹ That made macrophages (Mφ) of the ST suspect to synthesize PADs. Moreover, we demonstrated that among various polarised subsets, the M-CSF-Mφ present the highest pro-inflammatory response to ACPA-containing immune complexes.² Recently, we confirmed that PAD2 and 4 are expressed in monocytes and showed that PAD2 is expressed at various degrees in monocyte-derived polarised Mφs generated in the presence of IFN-γ, IL-4, IL-10 or M-CSF, while PAD4 is only detected in the IFN-γ Mφ.

Objectives To evaluate expression of PAD2 and 4 in Mφ polarised by M-CSF, activated by various polarising or pro-inflammatory cytokines, expressed in the ST.

Methods CD14⁺ monocytes from healthy donors were differentiated in Mφ in the presence of M-CSF and were subsequently exposed for 18 hour either to polarising cytokines or to pro-inflammatory cytokines such as TNF-α, IL-6, IL-8 or IL-17. *PADI2* and 4 gene expression was measured by RT-qPCR and expression of the related proteins evaluated by immunoblotting on total cell extracts.

Results *PADI2* mRNAs are less detected in M-CSF-Mφ than in the monocytes while *PADI4* gene expression is totally suppressed. All the polarised M-CSF-Mφ subsets retain *PADI2* gene expression, and interestingly, after IFN-γ stimulation *PADI2* mRNAs are detected at a higher relative rate. However IFN-γ, IL-4 or IL-10 does not induce *PADI4* gene re-expression. Whereas PAD4 protein remains undetectable, PAD2 is detected in all the polarised M-CSF-Mφ subsets but not more after IFN-γ stimulation.

M-CSF-Mφ activation by TNF-α, IL-8 or IL-17 does not modulate expression of the *PADI2* and 4 genes. IL-6 induces a significant decrease of *PADI2* gene expression and has no effects on the *PADI4* gene. Finally, the protein expression of PAD2 is not modulated in the various activated M-CSF-Mφs, and PAD4 is never induced.

Conclusions This study shows that in M-CSF-Mφ *PADI2* gene expression can be modulated by pro-inflammatory cytokines while *PADI4* gene expression cannot be re-induced. Our results reinforce the hypothesis of monocytes and Mφ involvement in generation of the ACPA epitopes in the ST of RA patients.

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Acknowledgements The technical assistance of C. Thomann, G. Offer and I. Belhaouane is gratefully acknowledged.

Disclosure of Interest None declared.

P009/O22

TRANSCRIPTOMICS UNVEILS UNIQUE BIOLOGICAL PROFILE OF TERTIARY LYMPHOID STRUCTURES GERMINAL CENTERS

¹S Colafrancesco*, ²E Pipi, ³J Campos, ³S Nayar, ²V Iannizzotto, ⁴R Priori, ⁵G Valesini, ³B Fisher, ²F Barone. ¹*Reumatologia, University of Rome Sapienza, Rome, Italy*; ²*RRG*; ³*University of Birmingham, Birmingham, UK*; ⁴*University of Rome Sapienza*; ⁵*University Sapienza, Rome, Italy*

10.1136/annrheumdis-2018-EWRR2019.6

Career situation of first and presenting author Post-doctoral fellow.

Introduction The development of the B cell repertoire is regulated by the process of affinity maturation that occurs within the inner part of the B cell follicles within secondary lymphoid organs (SLOs). In autoimmunity process might occur in ectopic lymphoid structures (ELS), aggregates of lymphocytes that form in target organs of disease (i.e. the salivary glands of patients with Sjogren's Syndrome (SS)). The phenotypical and functional features supporting ELS pathogenic properties have not been identified. Moreover, the functional proof that ELS independently from SLOs contribute to the autoimmune response has not been provided.

Objectives To characterise the transcriptome profile of human ELS isolated from SS salivary glands in comparison with SLO and to dissect, in an animal model of ELS development, the ability of ELS to contribute to the autoreactive response.

Methods Frozen salivary gland biopsies were obtained from SS patients and selected for presence of germinal center +ELS. Samples were stained and microdissected, RNA isolated and transcribed and used for RNAseq using ClonTech SMARTseq v4 kit.

Salivary gland ELS and autoimmunity was established as previously described¹ in FAP-DTR mice. Animals were locally treated (day 2 and 6) with DTX to induce selective ELS disaggregation; glands and serum were harvested for analysis at day 15.

Results Transcript analysis provide evidence that the ectopic ELS are characterised by upregulation of TNF, INFγ, BAFF, APRIL, CXCL12 and CXCL13, FAS and FASL as compared to SLOs. Sequencing unveiled an altered cell-proliferation profile with downregulation of BCL6 and AID, the enzymes responsible for B cell affinity maturation. Selective depletion of FAP +cells induced loss of anatomical segregation of ELS and profoundly compromised their anatomy with significant impact on the local autoimmune response.

Conclusions Our study provides the first evidence of a significant molecular difference between ELS and SLOs. We