P001

DIFFERENTIAL EXPRESSION OF KEY METABOLIC GENES IN ANTIGEN-SPECIFIC B CELL SUBSETS IN RHEUMATOID ARTHRITIS AND SYSTEMIC LUPUS ERYTHEMATOSUS

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

Introduction Antigen-specific B cells (Ag-Bs) differentiate into distinct subsets upon activation: plasmablasts (pBs, CD19⁺IgD⁺CD71⁺CD38⁺CD20⁻) and memory B cells (mBs, CD19⁺IgD⁺CD71⁺CD38^{dim}CD20⁺). The frequency of antigen-specific plasmablasts and memory B cells has been described to be altered in the blood of Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE) patients. Emerging evidence suggests that blood immune cells from RA and SLE patients exhibit different metabolic requirements and profiles when compared to healthy controls (HC). However, the metabolic configurations of these two Ag-Bs subsets in RA and SLE patients are yet to be described.

Objectives In the present study we aimed to characterize the gene expression of key metabolic enzymes in mBs and pBs from RA and SLE patients.

Methods Blood samples were obtained from 5 RA, 2 SLE and 3 HC donors. B cells were isolated and sorted into pBs and mBs populations. The gene expression of key metabolic enzymes was assessed by qPCR in both populations. The target genes analyzed were *HIF1A*, *HK2*, *LDHA*, *MYC* (glycolysis), *CPT1A* (fatty acid oxidation), *SREBF1* (lipid synthesis) and *PRKAA1* (mTOR inhibitor).

Results When comparing to HC there was a higher expression of: *HIF1A*, *LDHA*, *MYC*, *PRKAA1* in RA mBs; *HIF1A*, *HK2*, *LDHA*, *SREBF1* in RA pBs; *HIF1A*, *HK2*, *LDHA*, *CPT1A*, *SREBF1* in SLE mBs; *MYC* and *PRKAA1* in SLE pBs. When the gene expression was compared between RA and SLE patients, SLE mBs had a higher expression of *HK2*, *CPT1A* and *SREBF1* while SLE pBs exhibited higher levels of *MYC* and *PRKAA1* expression. Thus, the observed results suggest that RA mBs, RA pBs and SLE mBs exhibit a glycolytic profile when compared to HC. SLE mBs also seem to have higher fatty acid oxidation and lipid synthesis levels when comparing to HC and RA mBs. The higher expression of *SREBF1* in RA pBs could also indicate that lipid synthesis is upregulated in this subset. Interestingly, the results in SLE pBs suggest that all the analyzed metabolic pathways are downregulated when comparing to HC and RA pBs. Finally, since *PRKAA1* is upregulated in RA mBs and SLE pBs one might hypothesize that inhibition of mTOR is greater in these subsets.

Conclusions The size of our sample is the principal limitation in this study and therefore we aim to increase it. Nevertheless, these preliminary results seem to indicate that antigen-specific B cells exhibit marked glycolytic profiles in RA and SLE patients. These results are expected in RA given the highly glycolytic requirements of T cells in this disease. Our study demonstrates that B cell metabolism should be characterized in depth in both RA and SLE.

Disclosure of Interest None declared.

P002/O26

DIFFERENCE BETWEEN PALINDROMIC RHEUMATISM AND EARLY RHEUMATOID ARTHRITIS AT THE LEVEL OF GENE EXPRESSION

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Introduction Palindromic rheumatism (PR) is characterised by recurrent, episodic attacks of articular inflammation, which resolve completely without residual joint damage. Whether PR should be considered as a prodrome of rheumatoid arthritis (RA) or as a distinct syndrome remains unclear, over 70 years since first described.¹ PR patients (non flare and during flare) were recruited in Leeds; RA and HC data was obtained from the RA-Map consortium. Differentially expressed genes (DEGs) were identified following a standard microarray analysis workflow. These findings reported the presence of distinct gene expression profiles within palindromic patients, compared to HC and those with drug naïve, early RA.²

Objectives To validate a 10 gene signature as a discriminant between PR and RA in order to better classify patients suspected of having PR and to observe this signature as PR patients progress to early RA.

Methods PBMCs were taken from non-flare PR (n=24); flare PR (n=16); PR progressors to RA (PR→RA, n=10); RA (n=16); and patients suspected of having PR without an official diagnosis (SpPR, n=31). All patients provided informed written consent. qPCR gene expression analysis was performed using 10 TaqMan assays and a house keeping gene.

Results Functional analysis of the DEGs revealed significantly enriched gene pathways², from which a group of 10 DEGs with high expression fold differences between PR and RA were selected and grouped into three signatures: PR signature (high RPL10, low IL10, CCR6, UBC, and SHCBP1); RA signature (high ALPL, JUN and IFITM2); Non-Flare vs Flare signature (high EIF3E, low HSPA9).

Comparing PR (non-flare and flare) with RA, 2 of the 5 genes (IL10, UBC) associated with PR were significantly different to RA (p<0.05). For the RA signature, 2/3 genes (JUN and IFITM2) were expressed significantly higher in RA (p<0.01). Comparing PR→RA with RA, we observe higher levels of expression for 3/10 genes (ALPL, CCR6, SHCBP1, p=0.05), and overall higher median values for 7 genes, not including IFITM2 and IL10 (suggesting non-RA).

Finally, comparing SpPR to PR and RA groups, we observed little differences in gene expression with PR; suggesting a correct diagnosis as no patients displayed a signature resembling that of RA. Nevertheless, one gene (JUN) was significantly higher in SpPR compared with PR, suggesting these patients may be in a more progressed disease state when presenting at the clinic.

Conclusions There are distinct differences (at the level of gene expression) between PR and RA, and those patients who progress to RA from PR display a molecular signature that remains closer to PR than RA.

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P006

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

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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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P007

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

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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.