P035 THE TRANSCRIPTIONAL CO-ACTIVATOR BOB.1 PREVENTS TERMINAL DIFFERENTIATION AND INDUCES COSTIMULATORY CAPACITY OF B CELLS IN GC-LIKE ENVIRONMENT

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Introduction Recently we identified the transcriptional co-activator BOB.1 as specifically overexpressed in RA synovium, where its levels strongly correlated with the presence of germinal centres (GCs). In accordance with human data, mice lacking functional BOB.1 failed to mount GC response and were resistant to the experimental model of RA.
Objectives In this study we investigated whether increased levels of BOB.1 impact the phenotype and function of B cells in a GC-like environment.
Methods Expression levels of BOB.1 were assessed by qPCR and immunofluorescence. DNA encoding BOB.1 was introduced into B cells by retroviral-mediated gene transfer. The phenotype, activation and BCR signalling were analysed by FACS. Expression of CCL3 was measured by qPCR. Impact of high levels of BOB.1 in B cells on proliferative responses of autologous T_{FH} cells was assessed by FACS after 5 days of co-culture with SEB-pulsed B cells.
Results Immunofluorescence staining of inflamed RA synovium demonstrated the presence of high numbers of BOB.1-positive cells clustered in the lymphoid follicles in a pattern similar to the normal tonsillar tissue. Further analysis revealed that expression of BOB.1 in RA synovial B cells was significantly higher than in tonsillar B cells. To investigate how high levels of BOB.1 impact phenotype and function of B cells in a GC-like environment we overexpressed BOB.1 in human primary B cells cultured with CD40L and IL-21. In these conditions B cells rapidly differentiate into antibody-producing plasma cells, a process that is accompanied by a decrease in expression of CD20 and increase in CD38 and CD27. In contrast to the control, cells transduced with BOB.1 retained CD20 expression while expressed intermediate levels of CD27 and CD38. Accordingly, the percentage of plasmablasts was significantly lower in BOB.1-overexpressing cells, confirming that high levels of BOB.1 suppress plasma cell differentiation. Further analysis revealed that BOB.1-transduced memory B cells expressed high levels of costimulatory receptors CD40, CD80 and PD-L2 involved in B-T cell interactions and showed more rapid Ca^{2+}mobilisation and increased production of T-cell-recruiting chemokine CCL3 following BCR stimulation. Accordingly, T_{FH} cells co-cultured with BOB.1-transduced B cells exhibit a higher rate of proliferation as compared to T_{FH} cells co-cultured with B cells transduced with the control retrovirus.
Conclusions These data suggest that increased levels of BOB.1 in B cells during T cell-dependent responses in GCs suppress their terminal differentiation and enhance expression of costimulatory molecules and BCR signalling strength, which is translated into their greater help to T_{FH} cells. Whether this is sufficient to drive/accelerate autoimmune disease will be evaluated in a B cell-specific BOB.1 tg mouse model.
Disclosure of interest None declared

P036 NEUTROPHIL EXTRACELLULAR TRAPS IN SYSTEMIC LUPUS: A PROTEOMIC ANALYSIS

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Introduction An altered formation and/or removal of neutrophil extracellular traps (NET) has been recently proposed as key event in systemic lupus (SLE): in fact, NETs have been detected in the skin and kidney of SLE patients, low density granulocytes from SLE patients form more frequently NETs and NET degradation is impaired. However, no data are yet available on NET composition in SLE patients.
Objectives The aim of the study is to compare NETs from SLE patients and normals by means of proteomic analysis.
Methods Neutrophils from 11 normals and 16 SLE patients (9/16 with active disease) were stimulated by PMA, NET proteins were released in supernatants by DNase treatment and characterised by Fusion Orbitrap Mass spectrometry.
Results Overall, 802 proteins were detected (50% identified in all the samples); 236 are exclusively found in control NETs, 57 only in active SLE and 22 in inactive SLE. Multidimensional scaling (MDS) showed that NETs composition/amount allows the identification of 3 different clusters corresponding to active SLE, inactive SLE and controls in a bi-dimensional scatter plot. The heat map describing the proteome profiles shows two different clusters of proteins, one represented by 16 proteins that were more expressed in inactive disease, the other by 9 proteins more expressed in active SLE. A preliminary analysis of posttranslational modifications showed a differential expression of methyloxidized and phosphorylated proteins.
Conclusions The data obtained suggest that NET composition and type/extent of posttranslational modifications are different in active SLE, in inactive disease and in controls. The different cytokine and autoantibody environment that distinguishes SLE flares from inactive may activate different pathways in NET induction and condition the quality and quantity of NET-associated proteins.
Disclosure of interest None declared

P037 MULTIPLE ROLES OF PHOSPHOLIPASE C-ETA2, AS A NOVEL C2 DOMAIN CONTAINING PROTEIN, IN THE PATHOGENESIS OF RHEUMATOID ARTHRITIS

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Introduction The *C2 domain* is a Ca^{2+}-dependent membrane-targeting motif found in many cellular proteins involved in signal transduction or inflammation pathway. However, the effects of C2 domain-containing proteins in the fibroblast-like synoviocyte (FLS) of rheumatoid arthritis (RA) have not yet been elucidated.
Objectives The aim of this study was to screen novel C2 domain-containing proteins related to aggressiveness of FLS, and confirm the precise roles of target protein in RA.
Methods We transduced RA-FLS with a recombinant adenovirus expressing a C2 domain library. To confirm the effect of phospholipase C-eta2 (PLCH2), as a candidate C2 domain-