

additional experiments are needed to interpret these results. In future work we will compare IgG with known affinity to subclasses of CCP antigens to verify that our technique distinguishes these more subtle differences in epitope specificity. In addition, it will be investigated whether fine specificity may affect the progression to arthritis in ACPA+ arthralgia patients.

A5.26 REGULATION OF EXPRESSION AND FUNCTION OF NEGATIVE IMMUNOMODULATORY RECEPTORS IN B-CELLS: IMPLICATIONS FOR THE PATHOGENESIS OF SYSTEMIC LUPUS ERYTHEMATOSUS

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Background and Objectives Fine tuning of B-cell activation and differentiation depends on convergent signals from the B-cell receptor (BCR), costimulatory/coinhibitory membrane receptors, and Toll-like receptors. We sought to examine the expression and function of the coinhibitory receptors programmed death-1 (PD-1), PD-1 ligand-1 (PD-L1), B and T lymphocyte attenuator (BTLA) in B-cells from healthy donors, and from patients with systemic lupus erythematosus (SLE), the prototype of systemic autoimmune disease characterised by activated B-cells and production of high-titer autoantibodies by long-lived plasma cells.

Materials and Methods Peripheral blood CD19⁺ B cells were purified from healthy donors (n = 11) and active SLE patients (n = 15; SLE disease activity index 8.3 ± 2.7 [mean \pm SEM]). PD-1, PD-L1, and BTLA were examined by flow cytometry in naïve (CD19⁺CD27⁻), memory/transitional (CD19⁺CD27⁺), and plasma B-cells (CD19⁺CD27^{hi}) at baseline and following stimulation. Activation, differentiation, and proliferation (CFSE dilution) of B-cells were examined in the presence or absence of the BTLA ligand, HVEM. Western blot was used to assess the phosphorylation of intracellular kinases.

Results In healthy donors, the coinhibitory receptors PD-1 and PD-L1 were significantly upregulated on circulating plasma-cells compared to transitional/memory and naïve B-cells (PD-1: $36 \pm 7\%$, $14 \pm 3\%$, $2.0 \pm 0.5\%$; PD-L1: $94 \pm 2\%$, $83 \pm 5\%$, $62 \pm 8\%$, respectively, $p < 0.001$). BTLA was expressed by 93–100% of B-cells, and mean fluorescence intensity was significantly higher in plasma-cells (334 ± 146 versus 127 ± 12 in naïve B-cells, $p = 0.048$). BCR activation enhanced the expression all three receptors in normal B-cells; addition of CpG-ODN (TLR-9 ligand) further induced PD-1 and PD-L1-but not BTLA-expression, whereas addition of the cytokines IL-4, IL-10, or IL-21 reduced PD-1 and BTLA levels. In vitro crosslinking of BTLA resulted in reduction of BCR-induced phosphorylation of ERK, CD80/CD86 and BAFF-receptor expression, as well as in inhibition of cell proliferation (divided cells: $5.3 \pm 0.4\%$ versus $17.7 \pm 0.1\%$ in anti-IgM-stimulated cells). In comparative analysis, SLE patients exhibited significantly higher PD-1 expression on plasma-cells compared to healthy donors ($65 \pm 5\%$ versus $36 \pm 7\%$, $p = 0.002$), whereas there was no difference in PD-L1 or BTLA. Preliminary studies suggest distinct roles for PD-1 and BTLA in regulation of activation and maturation of B-cells in healthy controls and in the context of lupus.

Conclusions The coinhibitory receptors PD-1, PD-L1 and BTLA demonstrate differential expression among B-cell subsets and they are induced upon stimulation with important implications for the regulation of B-cell activation, proliferation and differentiation. Aberrancies in the expression and function of coinhibitory receptors in SLE plasma B-cells could contribute to enhanced autoantibody-forming capacity and disease pathogenesis.

A5.27 Ro52 EXPRESSION IS A PROGNOSTIC FACTOR FOR SURVIVAL IN B CELL LYMPHOMA

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Background and Objectives The risk for lymphoma is increased in many rheumatic conditions. Diffuse Large B-cell Lymphoma (DLBCL) is a common, yet heterogeneous lymphoma subtype and good prognostic biomarkers are warranted. We have previously shown that the rheumatic autoantigen Ro52 is expressed predominantly in leukocytes and that over expression affects lymphocyte proliferation. Further, the Ro52 (*Trim21*) gene maps to a tumour suppressor locus. We therefore hypothesised that Ro52 expression might be used as a prognostic marker for lymphoma.

Materials and Methods Proliferation of in vitro stimulated lymphocytes was determined by thymidine incorporation and cell cycle analysis and compared to Ro52 mRNA levels. Three cohorts of patients with DLBCL were investigated for Ro52 expression by immunohistochemistry using monoclonal Ro52 antibodies. The first cohort consisted of patients with rheumatic disease treated with cytostatic drugs only (n = 43). The two others were non-rheumatic patients treated with anthracycline-based (CHOP) therapy alone (n = 74), and with the addition of Rituximab (n = 196).

Results A robust inverse correlation between Ro52 expression levels and cellular proliferation was observed ($r^2 = 0.50$, $p < 0.0001$). Confirming this biological phenomenon, low expression of Ro52 in lymphoma tissue was significantly correlated to overall survival ($p < 0.0001$), as well as progression-free survival ($p < 0.0001$) in patients with DLBCL treated with CHOP therapy alone. This was further confirmed in the Rituximab-CHOP treated cohort, with a significantly lower overall survival ($p < 0.0001$) and progression-free survival ($p = 0.0005$). The association was independent of Ann Arbor classification and DLBCL subtype, emphasising that Ro52 expression is a parameter of added value in lymphoma investigations to understand the patient prognosis.

Conclusions Our data demonstrate that low Ro52 expression is associated with more aggressive lymphomas. Further, our data functionally link low Ro52 expression with increased cellular proliferation. Ro52 may thus constitute a novel biologically relevant biomarker predicting patient survival in lymphoma.

A5.28 SEMAPHORIN3A IS A POTENT B CELL REGULATORY MOLECULE IN SLE

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Background/Purpose Semaphorin3A (sem3A) is an important regulatory molecule, previously reported to play a role in the pathogenesis of rheumatoid arthritis and later by us in lupus glomerulonephritis. In addition, sema3A was shown to be a marker of T regulatory cells. A subpopulation of B cells, namely B regulatory cells (Bregs), was recently identified by us as CD19⁺CD25^{high}IL-10^{high}TGF- β ^{high}, and demonstrated to be of high sema3A expression.

We therefore asked whether serum levels of sema3A is altered in SLE patients, whether sema3A expression on B regs of these patients is different, and finally, whether the addition of soluble sema3A to