AI-04 HIGH TITER ANA NOT NECESSARILY A VALID CRITERION FOR LUPUS – PROPOSAL OF A MODIFICATION TO THE CRITERIA FOR CLASSIFICATION OF SLE

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Background The 1997 Update of the 1982 American College of Rheumatology Revised Criteria for Classification of SLE includes two autoantibody criteria: #10, abnormal level of anti-native DNA, anti-Sm, or antiphospholipid; #11 positive antinuclear antibody (ANA). Thus, ANA positivity is counted as 1 of the 11 criteria and a person shall be said to have SLE if *any 4 or more of the 11 criteria are present, serially or simultaneously, during any interval of observation*. The immunofluorescence pattern observed in the ANA test provides a direct initial assessment of ongoing autoantibody response in candidate patients of many systemic autoimmune rheumatic diseases (SARD). As a follow-up to the International Consensus on ANA Patterns (ICAP) initiative (ANApatterns.org), which aims to promote harmonisation of ANA pattern nomenclature and provides guidelines for ANA interpretation, thereby optimising usage in patient care, the relevance of each ANA pattern is being re-evaluated.

Methods Collective issues on ANA nomenclature were raised among research, clinical, and diagnostic laboratories represented by two workshop participants and a working committee. Post-workshop exchanges arrived at consensus on a few, but clearly not all, issues. One focus is to establish an interpretative clinical description for each defined ANA pattern for clinical use based on current literature.

Results Consensus was achieved for 28 ICAP patterns designated with alpha-numeric code (AC-1 to AC-28) and summarised under a nomenclature and classification tree categorised in three major groups (nuclear, cytoplasmic, and mitotic patterns). An important observation is that, while the Homogeneous (AC-1) and Coarse Speckled nuclear (AC-5) patterns are linked to autoantibodies strongly associated with SARD, the Dense Fine Speckled (DFS) nuclear pattern (AC-2) virtually rules out a SARD diagnosis. A clear cut DFS pattern is usually present when anti-DFS70/LEDGF/P75 is the only predominant autoantibody in the serum sample. DFS is the most common pattern in high titer ANA-positive, apparently healthy, individuals. Although DFS has been reported in a wide variety of chronic inflammatory diseases, such as Hashimoto's thyroiditis, atopic dermatitis, interstitial cystitis, Vogt-Koyanagi-Harada syndrome, and in miscellaneous non-inflammatory diseases, it is not associated with SARD, even when present at very high titer.

Conclusions ICAP has clearly provided a common platform to address issues that are of great interest to the ANA community and closely linked to ANA in disease criteria. Evidently, well-defined anti-DFS ANA, confirmed by antigen-specific reflex testing, should not be considered a criterion for SLE – either in the ACR or 2012 SLICC classification criteria.

AI-05 RESPONSE GENE TO COMPLEMENT-32 PROMOTES PLASMA CELL DIFFERENTIATION AND ENHANCES LUPUS-LIKE CHRONIC GRAFT VERSUS HOST DISEASE

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Background Response Gene to Complement (RGC)−32 is an intracellular protein that plays a role in cell growth and promotes cell cycle activation and Akt phosphorylation. RGC-32 is also a downstream target of TGF-β in fibroblasts and renal proximal tubular cells and plays a role in renal fibrogenesis. In immune cells, RGC-32 is expressed by both T and B lymphocytes. Our prior studies showed that RGC-32 promotes Th17 differentiation of mouse CD4 T cells and is highly expressed in human IL-17 CD4 cells. Whether RGC-32 plays a role in the activation and differentiation of B cells and the development of autoimmunity is not known. We used WT and RGC-32 KO mice to determine whether lack of RGC-32 impairs B cell differentiation and activation and alters autoimmune parameters in the chronic graft versus host disease (cGVHD) model of lupus.

Materials and methods B cells were cultured with lps, anti-CD40 mAb, IL-21 and IL-6, IL-4 or TGFβ and RGC-32 mRNA and protein expression was determined. TLR-dependent and T dependent B cell differentiation to plasma cells (PC) was induced with lps and with CD40mAb plus IL-4. cGVHD was induced with 100×10^6 Bm12 splenocytes injected into WT or RGC-32 KO recipients. Host B cell number and activation, anti-dsDNA Ab production, germinal centre (GC) B cell number and proliferation, PC number, expression of transcription factors IRF4 and Blimp1 were assessed at 2 and 4 weeks.

Results RGC-32 mRNA was upregulated in B cells by lps, anti-CD40 mAb, IL-21 and IL-6. RGC-32 KO B cells failed to differentiate normally to PC as demonstrated by a 2-fold reduction in PC numbers generated after lps and anti-CD40+ IL-4 stimulation and impaired upregulation of Prdm1 and IRF4 mRNA. RGC-32 transcripts were upregulated in spleen cells from cGVHD mice and protein expression was detected in B cells and GC cells. RGC-32KO hosts displayed an attenuated autoimmune phenotype as demonstrated by: 1) decreased production of anti-dsDNA autoAb. 2) decreased number and proliferation of GC B cells. 3) decreased number of IgG anti-dsDNA secreting PC and 4) decreased IRF4 and Prdm1 mRNA expression.

Conclusions These results suggest that expression of RGC-32 in B cells is critical for optimal GC proliferation, PC differentiation and autoantibody production in a murine model of lupus. These data support the idea that RGC-32 blockade has the potential to attenuate autoimmune parameters of cGVHD and possibly reverse abnormalities in the T and B cell pathways that contribute to lupus pathogenesis.

AI-06 DALAZATIDE, AN INHIBITOR OF THE KV1.3 CHANNEL ON ACTIVATED EFFECTOR MEMORY T CELLS, HAS IMMUNOTHERAPY POTENTIAL AGAINST SYSTEMIC LUPUS ERYTHEMATOSUS

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Background T cell activation depends upon a calcium signalling cascade that is regulated by voltage-gated potassium channels. Effector memory T cells (T_{EM}), which are implicated in the immunopathogenesis of autoimmune diseases, express relatively high levels of the potassium channel Kv1.3. Dalazatide is a potent peptide inhibitor of the Kv1.3 channel that has recently shown safety and efficacy in a Phase 1 b plaque psoriasis trial. Evidence suggests that inflammatory cytokine producing T_{EM} cells might be involved in the immunopathology of lupus nephritis. The objective of this study is to provide proof-of-principle *ex vivo* data for therapeutically targeting chronic T cell activation in systemic lupus erythematosus (SLE).
Materials and methods Peripheral blood mononuclear cells from paediatric and adult SLE patients as well as healthy controls were studied. T lymphocyte subsets were assayed *ex vivo* for Kv1.3 expression by flow cytometry. The effect of dalazatide on inflammatory cytokine expression by T_{EM} cells activated by thapsigargin/phorbol myristate acetate (PMA) or ionomycin/PMA was evaluated by intracellular cytokine staining.
Results Kv1.3 expression by $CD8^+$ T_{EM} cells was significantly higher in patients with active lupus nephritis when compared to patients with inactive SLE or healthy controls. Dalazatide inhibited IFN-γ, IL-17 and TNF-α production by both $CD4^+$ and $CD8^+$ T_{EM} cells from SLE patients in a dose-dependent manner. Dalazatide-mediated inhibition was more significant in IFN-γ and TNF-α-expressing $CD4^+$ T_{EM} cells from patients with active SLE compared to cells from patients with inactive disease.
Conclusions *Ex vivo* studies suggest that dalazatide inhibition of Kv1.3 on T_{EM} may be an effective strategy for treating SLE. In addition, Kv1.3 expression may be a useful biomarker of SLE disease activity.

AI-07 INCREASED "PROBLEMATIC" RNA SPLICING AND GREATER INTRON NUMBER IN SLE AUTOANTIGENS

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Background Proteins arise from splicing of pre-mRNA using either the U2 spliceosome (most proteins); or the U12 spliceosome. The latter is believed to be less efficient, and it has been reported that autoantigens are much more likely than control proteins to use the U12 spliceosome. We set out to extend this work, and using new databases and a better contemporary understanding of splicing, to address the splicing mechanisms used for commonly encountered autoantigens.
Materials and methods We compared splicing characteristics of the UniProt autoantigen database with total genomic proteins, using the approach of Parada *et al*. (*Nucleic Acids Res. 42:10564, 2014)*. Using this method, splice sites are given a "fit" score reflecting their fit to canonical scoring matrices for the two types of introns. We also determined the number of introns per gene and the average intron length.
Results We confirmed that autoantigens had more U12 spliceosome usage, although the difference was only twofold, much less than in the one previous study (Ng *et. al*., *J All Clin Immunol 114:1463, 2004)*. Autoantigens had increased average number of introns per gene (24 vs 12) and an increase in noncanonical dinucleotides at the splice site. When they were scored for "problematic" splices, autoantigens were three fold more likely to have problematic introns (39% vs 13%).
Conclusions Genes encoding autoantigens have more introns and more "problematic" introns than control proteins. This may result in greater numbers of splicing errors, giving rise to proteins toward which tolerance has not been established. This model predicts autoantigenic epitopes to be near splice sites and should encourage studies of more extensive databases of autoantigens to extend analysis.
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AI-08 B CELL PHENOTYPIC CHANGES IN ANTI-NUCLEAR ANTIBODY POSITIVE INDIVIDUALS PRIOR TO THE ONSET OF SYSTEMIC AUTOIMMUNE RHEUMATIC DISEASE

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Background Patients with systemic autoimmune rheumatic diseases (SARD) often have a prolonged pre-clinical phase during which they are anti-nuclear antibody $(ANA)^+$ but lack clinical symptoms. Here we sought to determine whether ANA^+ individuals who lack sufficient symptoms for a SARD diagnosis share the B cell phenotypic changes seen in SARD.
Materials and methods Healthy controls (HC) and ANA^+ individuals who: 1) lacked clinical symptoms of SARD (ANS); 2) had at least one clinical symptom of SARD (UCTD); or 3) had recently diagnosed steroid and immunosuppressive naïve SARD (SLE, SS, SSc, MCTD, DM) were recruited. PBMCs were stained with various combinations of fluorescently labelled antibodies and analysed by flow cytometry. Anti-nuclear antibodies were measured through the hospital laboratory. Whole blood IFN signature and BAFF RNA levels were measured by NanoString.
Results B cell phenotypes were examined for 32 HC, 38 ANS, 28 UCTD, and 59 early SARD patients. Patients with early SARD had a number of changes in their naïve and memory B cell subsets including: increased proportions of mature naïve (SSc) and T1T2 cells (SLE and SS), and decreased proportions of switched memory cells (all SARD). Similar decreases in the proportion of switched memory B cells were seen in ANS and UCTD patients, and as seen for the SARD patients, these cells were activated with elevated levels of CD86 as compared to HC. Significantly increased activation of the $CD27^-IgD^-$ memory compartment was also seen in ANS, UCTD, SLE and SjD patients. Although significantly increased proportions of plasmablasts and/or $CD138^+$ plasma cells were seen in early SARD patients, these were not seen in ANS and UCTD patients. Nevertheless, in pre-SARD individuals (ANS + UCTD) there was a significant positive correlation between the size of these cell subsets and ANA titer as well as the number of different anti-nuclear antibody specificities. As observed for early SARD patients, there was a trend to increased BAFF levels as compared to HC in pre-SARD individuals, which achieved statistical significance in UCTD patients. However, there was no association between the levels of BAFF and any of the B cell phenotypes, whereas the IFN signature was positively associated with the proportion of T1T2 cells.