### Abstracts

**AI-11**  
**ISOLATING AND CHARACTERISING AUTOACTIVE T CELLS IN LUPUS**  
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10.1136/lupus-2016-000179.11

**Background** Activation of autoreactive T cells is a critical step in the pathogenesis of lupus. These T cells help autoreactive B cells, make inflammatory cytokines, and infiltrate tissues. Yet, compared to autoreactive B cells, little is known about the specificity, identity, origins, or functions of autoreactive T cells. Therefore, we sought to isolate, clone and characterise T cells that recognise peptides derived from the targets of anti-nuclear antibodies/B cells.

**Materials and methods** Using anti-IgG2a ("rheumatoid factor", RF) B cells from site-directed transgenic mice as "universal APC" for the contents of dying cells that are in turn bound by IgG2a anti-nuclear antibodies (ANAs). We relied on a monoclonal IgG2a anti-chromatin to stimulate the T cells initially and then a panel of monoclonal ANAs to test them. These ANAs naturally form immune complexes (ICs) with material released from dead cells either in vitro or in vivo. We used these tools to serially stimulate primary polyclonal T cells and make autoreactive T cell hybridomas. We then cloned the TCRs of these hybridomas into retroviral vectors to make "recombinant mice", a source of primary T cells.

**Results** We isolated multiple clones of T cells that could help RF B cells make proliferate and differentiate both in vitro and in vivo and characterised two in detail. These T cells were activated by the combination of RF B cells and either IgG2a anti-chromatin or anti-RNA, indicating that the T cells recognised peptides derived from the ICs formed by the ANAs used to stimulate them. We further found that while TLR7 and 9 were required to stimulate the RF B cells in the absence of T cells, the requirement for these molecules was bypassed by the presence of autoreactive T cells. Reciprocally, the APC function of B cells for T cells also did not require TLR7/9 expression in B cells.

**Conclusions** We used a novel method to clone and characterise autoreactive T cells that help autoreactive B cells. These T cells were isolated from normal animals without use of adjuvant or foreign Ag, confirming that normal primary repertoires contain relevant autoreactive T cells. These cells enhanced multiple modes of B cell activation and differentiation in vivo and themselves were activated and differentiated in divergent ways. Most importantly, because they could bypass in large part the need for B cell-intrinsic TLR stimulation, they support the idea that TLRs may be more important for initiation of autoimmunity rather than propagating it once it is well-established.

**Acknowledgements** Supported by a grant from the Lupus Research Institute.

**AI-12**  
**GLOMERULAR-TARGETED INHIBITION OF PROTEIN KINASE C-α AMELIORATES ANTIBODY-MEDIATED NEPHRITIS BY RESTORING MITOCHONDRIAL DYSFUNCTION IN ENDOTHELIAL CELLS**

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10.1136/lupus-2016-000179.12

**Background** Lupus nephritis is a major cause of morbidity and mortality that affects over half of the SLE population during the course of disease. Multiple factors contribute to the diverse nature of nephritis. Nevertheless, PKC-α activation, with consequent synthesis of TGF-β and matrix constituents, are common mediators of disease progression. In a murine model of antibody-mediated nephritis, nophritic nephritis (NTN), that simulates the effector phase of lupus nephritis, we investigated the role of targeted PKCα inhibition on progressive renal injury.

**Materials and methods** After induction of nephritis, on day 2, mice were treated with either: (1) nothing, (2) the PKCα inhibitor (Ro-32-0432) i.p., or (3) the PKCα inhibitor conjugated to F1.1, a human monoclonal α3(IV) collagen antibody that targets glomeruli. Resolution of disease was determined serologically and by histology. Comparative proteome analysis, from kidney cortices, was performed using quantitative mass spectrometry.

**Results** On day 7, the untreated NTN mice had severe nephritis (by BUN, proteinuria and, histology), whereas mice that received PKCα inhibitors, in either form, had minimal evidence of kidney injury: BUN was reduced to 32.29 ± 1.70 mg/dL and 36.07 ± 4.51 mg/dL in PKCα inhibitor and F1.1- PKCα inhibitor injected mice vs. 99.84 ± 0.33 mg/dL in NTN mice, and there was a parallel normalisation of histology. Serum cytokine levels confirmed reduction of systemic inflammation. To further define the role PKCα inhibition, comparative proteome analysis from kidney cortices was performed. The functional protein groups most affected by NTN were mitochondrial proteins associated with respiratory processes; proteins of all four complexes of the respiratory chain were down regulated in NTN mice. By contrast, their expression was restored by PKCα inhibition, suggesting a role for proteins that regulate oxidative phosphorylation in recovery. This hypothesis was further evaluated in glomerular endothelial cells by measuring respiration (oxygen consumption rates, OCR), and glycolytic lactic acid production (ECAR) with injury and recovery. NTS reduced OCR (223 pmol/min to 185 pmol/min) and increased ECAR (42 to 62 μM/mM/min). PKCα inhibition normalised OCR and ECAR levels, and this was associated with restoration of cellular function.

**Conclusions** The results suggest that PKCα is an important mediator of glomerulonephritis. Furthermore targeted inhibition of this enzyme protects the kidneys from progressive damage associated with severe inflammation by restoring oxidative phosphorylation. This has therapeutic implications for treatment of human glomerular diseases, including lupus nephritis.

**Acknowledgements** Istvan Czikota is acknowledged for his technical contributions.

**AI-13**  
**PROTEIN MODIFICATIONS THAT TRIGGER T CELL AUTOIMMUNITY**

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10.1136/lupus-2016-000179.13

**Background** T cell responses are known to be amplified in SLE, via TCR pathways of signal transduction. In particular, increases in intracellular tyrosine phosphorylation, calcium flux and actin polymerization are among the abnormal responses found in SLE T cells. An imbalance of how T cells signal through both Syk and ZAP70 shape the activation and cytokine responses in human SLE. The present study examines posttranslational protein modifications (PTMs) that arise in the context of SLE inflammation.
PTMs are known to alter both immune tolerance to self proteins (such as citrulline PTMs that are diagnostic in RA) as well as intracellular metabolic and signalling pathways. In particular, isoaspartyl (isoAsp) modification is one intracellular PTM previously demonstrated to be increased by cellular stress and inflammation. The present study examined T cell biology that is altered by PTMs in lupus.

Materials and methods Isoaspartyl PTMs were characterised in lymphocytes from both human SLE and in murine models. We specifically examined ZAP70 for PTMs to determine effects on intracellular signalling and cytokine production. We also examined ZAP70 amino acids sequences prone to isoaspartyl modification under inflammatory stress and their role in p-Tyr signal transduction, effects on downstream functional domains, and binding to cbl-b.

Results PBMCs from SLE patients and from MRL lupus both have elevated levels of intracellular isoaspartyl modifications and hyperproliferative T cell responses. We identified 4 specific sites of isoAsp modification (Figure 1), two within the I-B functional domain of ZAP70, including the c-Cbl, Vav and Lck binding domain. IsoAsp modified ZAP70 reduces c-Cbl binding, upregulates TCR and T cell hyperplasia. Enzymatic repair of intracellular isoAsp modification corrects T cell hyperproliferative defects that are characteristic of murine and human SLE.

Conclusions This study has examined mechanisms of altered T cell autoimmunity in SLE. In particular, abnormal T cell hyperproliferation was found to be a result of isoaspartyl modification at 4 specific sites within ZAP70. Only a small number of PTMs are known to arise in the context of inflammation. Our study suggests that SLE is characterised by an inability to control or repair excessive production of PTMs due to inflammation, leading to altered cell biologic functions, specifically T cell hyperproliferation. Physiologic repair of intracellular isoAsp modifications reversed abnormal proliferative T cell responses and may provide one therapeutic pathway for intervention.

Acknowledgements This study is supported by NIH AI48120.