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, nephritic 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 normalization of histology. Serum cytokine levels confirmed reduction of systemic inflammation. To further define the role PKCα inhibition, comparative proteome analysis from kidney cortices were 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 lactate acid production (ECAR) with injury and recovery: NTS reduced OCR (223 pmol/min to 185 pmol/min) and increased ECAR (42 to 62 mPµH/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.
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 acid 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 modifications 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.

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